SEXUAL DIFFERENCES OF THE LIVER CELLS IN THE GOLDFISH, *CARASSIUS AURATUS* L.

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It is well known that the liver of various fishes undergoes seasonal variations in size and in the content of fat and glycogen (Olivereau & Leloup, 1950; Pickford, 1953; Ito *et al.*, 1962). Moreover, it has been observed that, in some teleosts, the liver of female fishes is larger in size and weight than that of males especially in their spawning season (Amemiya & Tamura, 1948; Noguchi & Bito, 1953). However, only a few histological studies are concerned with sexual differences in the liver cells of mature teleost fishes (Kobayashi, 1953, in *Misgurnus anguillicaudatus*; Egami, 1955, in *Oryzias latipes*; Oguro, 1956, in *Gasterosteus aculeatus aculeatus*). The female sex seems to be responsible for the sexual differences of the liver cells in these species, for the differences can be abolished either by ovariectomy of mature females or by the administration of estrogen to mature males.

Electron microscopic studies of the liver cells of fishes have been done by some investigators (Yamamoto, 1964; Kitada & Takagi, 1965; Berlin & Dean, 1967). So far as the writers know, however, no information from the ultrastructural point of view has been reported of the sexual differences of the liver cells of fishes.

The present study is to investigate light and electron microscopically the sexual differences in the liver cell and the effects of gonadectomy and of an estrogen on the cell of the goldfish, *Carassius auratus* L..

Material and Methods

In the present study, three series of experiments were carried out using the goldfish, *Carassius auratus* L., as material. In the first series, 10 mature fish of both sexes were reared in glass aquaria from April 20 until May 19, the period when they were expected to perform actual breeding. They were kept at a constant temperature of 20°C and were fed on commercial pellets of trout diet. At the end of this rearing period, the fish were sacrificed for the sake of morphological studies on liver cells. In the second series, 8 mature females were bilaterally ovariectomized on April 10, and 2 of them were killed 12 days later and 2 others 20 days after the operation. Two out of the remaining 4 fish were fed on the pellets containing ethinylestradiol at a dose of 100 μg/g diet during 14 days starting from the 30th postoperative day. The remaining 2, which were raised on the normal diet, served as controls. At the end of the hormone treatment, the control and

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treated fish were sacrificed. In the third series, 2 groups of immature goldfish about 100 days old were each treated with ethinylestradiol (100 μg/g diet) and methyltestosterone (25 μg/g diet), respectively, for 15 days beginning on October 1. At the end of the hormone treatment, 7 estrogen-treated and 3 androgen-treated fish were sampled for histological study on the liver.

For light microscopic observations, the liver was fixed with Bouin's fluid. Serial paraffin sections of the liver were cut 5–6 μ in thickness, and stained with Delafield's hematoxylin and eosin. In some cases, 1 μ sections of the material embedded in Epon for electron microscopy were stained with methylene blue-azur II mixture (Richardson *et al.*, 1961), and examined light microscopically for a comparison with pictures taken by electron microscope.

For electron microscopic observations, small pieces of the liver were fixed in Millonig's solution (Millonig, 1961) for two hours, dehydrated by graded ethanol and two changes of n-butyl glycidyl ether and embedded in Epon (Luft, 1961). Sections were cut with glass knives at 500–800Å in thickness on a Porter-Blum microtome, stained with uranyl acetate in combination with lead citrate, and examined with a Hitachi HS–7 electron microscope.

Results

The cytoplasm of the liver parenchymal cells in immature goldfish was usually stained weakly with eosin except in the perinuclear zone where the cytoplasm was stained darkly with hematoxylin, being furnished with a light zone in the peripheral region of the cell (Fig. 1). In the dark perinuclear zone of the cells, electron microscopically, there were found many cytoplasmic organelles such as mitochondria, Golgi complexes, endoplasmic reticulum and lysosomes. The mitochondria had a dense distribution around the nucleus, though some of them were found buried in the mass of glycogen granules in the peripheral light zone. The mitochondria were various in shape, viz., round, oval, short and long, rod-shaped, or gourd-shaped. A large amount of rough endoplasmic reticulum appeared as stacks of flattened cisternae in the vicinity of the nucleus. Similar granular reticulum was also found surrounding the mitochondria. Golgi complexes were observable most frequently around the intracellular bile capillary, though they were situated also in other parts of the cytoplasm. Lysosomes existed rather dispersedly in the cytoplasm (Fig. 10). No notable differences could be detected in the cytological characteristics of the liver cells between male and female in immature state.

In sexually matured fish, marked differences between the two sexes could be recognized in the histology of the liver cells. The cells of female livers fixed with Bouin's fluid were stained strongly with hematoxylin, with a roundish nucleus

containing a single, large nucleolus in the center (Fig. 2). In these cells, there appeared a lot of rough endoplasmic reticulum in the cytoplasm. They showed large stacks of flattened cisternae and often appeared in the peripheral portion of the cell. The glycogen deposit was less in the cells of mature females than those of mature males or of immature fishes (Fig. 11). No differences were detectable in the content of fat droplets between the mature male and the female liver.

On the contrary, the parenchymal cells of the mature male liver were generally larger in size than those of the female ones, and their nuclei were elliptical in shape in many cases. Moreover, the cells were less intensely stained with hematoxylin, displaying a reticular feature in the cytoplasm (Fig. 3). Ultrastructurally, the liver cells of the males had poorly developed an endoplasmic reticulum and an abundant glycogen deposit in the cytoplasm (Fig. 12). These characteristics of the liver cells of mature males were very similar in appearances to those of immature fish.

In the female fish from which mature ovaries had been removed, liver cells showed a progressive reduction in size following the operation. Forty-five days after ovariectomy, the nucleus of these cells appeared to be small, and the cytoplasm was stainable only in the perinuclear region (Fig. 4). In these cells, there was no prominent development of the rough endoplasmic reticulum but much deposit of glycogen granules in the cytoplasm. Accordingly, the liver cells of mature females had been returned to the state seen in those of immature fish by ovariectomy (Fig. 13). In ethinylestradiol-treated, ovariectomized fish, the liver cell showed conspicuous alterations in cytological features. The nucleus of these cells showed a hypertrophy and the cytoplasm became to be packed with many characteristic stripes which were stained strongly with hematoxylin (Fig. 5). Electron microscopically, there were a distinct depletion of glycogen deposits and a prominent increase in amount of the rough endoplasmic reticulum. The stripes found in the cytoplasm by light microscope seemed to be ultrastructurally the stacks of rough endoplasmic reticulum. A vesicular endoplasmic reticulum of small size also increased in amount in the cytoplasm. The mitochondria in the cells of treated animals became larger in size than in those of the control ones, and some of them showed vacuolization and/or a decrease density of the mitochondrial matrix. In addition, many dense bodies of various sizes, with peculiar myelinated structures, appeared scattered in the cytoplasm of the cells of the treated fish, while no such bodies were encountered in those of controls (Figs. 14 and 15).

Similar cytological modifications were also induced in the liver cells of immature fish, irrespective of their sexes, by the administration of ethinylestradiol (Figs. 6 and 7). On the other hand, administration of methyltestosterone to immature fish of both sexes failed to induce, light microscopically, any changes of the liver cell (Figs. 8 and 9), which retained the cytological characteristics seen in normal immature goldfish.

Discussion

It has been observed in several teleost species that the liver is different in cytological features of the parenchymal cells between the two sexes in the spawning season. Kobayashi (1953) showed that, in the loach, *Misgurnus anguillicaudatus*, the liver cells of the female were smaller in size but had larger nuclei with prominent nucleoli than those of the male. Egami (1955) reported in the medaka, *Oryzias latipes*, that the liver cells were larger in the female than in the male in the spawning season. Oguro (1956) noticed that, in the three-spined stickleback, *Gasterosteus aculeatus aculeatus*, the liver cells of the male were larger in size and contained a larger amount of fatty droplets than those of the female. In the loach, however, Kobayashi (1953) could not detect sexual differences in the fat content.

In the goldfish, *Carassius auratus*, it was evidenced by the present study that sexual differences of the liver cells occurred at least in the period of sexual maturation. In sexually immature fish, irrespective of their sexes, the liver cells had clear cytoplasm with an irregular reticular structure following the fixation with Bouin. After the sexual maturation of the fish had progressed, the cells began to show some changes in cytological features only in the female: they were smaller in size and had more roundish nuclei than those of the male; they were provided with darker cytoplasm without the reticular structure which was still prominent in the cells of the male. These sexual differences were ultrastructurally demonstrated by those in the amount of glycogen deposit and the degree of development of the rough endoplasmic reticulum in the liver cells. In the male, the cell had much more glycogen deposit whereas the rough endoplasmic reticulum was less developed in amount than those of the female. As mentioned before, the sexual differences found in the liver cells of the three-spined stickleback are attributable to the fact that the liver cells of the male contained a larger amount of fat droplets than those of the female (Oguro, 1956). In the present study, however, no ultrastructural differences were detectable in the amount of fat droplets in the liver cells between female and male goldfish. This finding on the fat droplets of the liver cells is in agreement with that reported by Kobayashi (1953) in the loach. The difference between the observations made by Oguro and by the present writers may be due to the difference of the species used or, more likely, to the fat content in the food given to the fishes, as suggested by Noguchi & Bito (1953).

In the medaka, Egami (1955) found that the livers of gonadectomized females came to resemble those of the males in histological structure, while the liver of castrated males remained almost unaffected. In mature goldfish, too, the liver cells showed a gradual reduction in size following ovariectomy. The nucleus appeared to be small, and the cytoplasm was stainable only in the perinuclear region in the liver cells of the ovariectomized fish. Accordingly, by ovariectomy,

the liver cells of mature females have been returned to the state seen in those of immature fish, thus exhibiting little difference in cytological aspects from those of mature males. Ovarian hormones seem to be responsible for the sexual differences of the liver cells.

In fact, ethinylestradiol-treated, ovariectomized goldfish showed conspicuous alterations in liver cells, which became to have close resemblance to those of normal mature female fish. In the affected liver cells, there were a distinct depletion of glycogen deposit and a prominent increase in amount of the rough endoplasmic reticulum, which were characteristic of the cells of the normal mature fish. Similar cytological modifications were also induced in the liver cells of immature fish, regardless of sex, by the administration of ethinylestradiol. On the contrary, the administration of methyltestosterone to immature fish of both sexes failed to induce any changes of the liver cell. Quite similar cases have been reported in the loach (Kobayashi, 1953), in the medaka (Egami, 1955) and in the three-spined stickleback (Oguro, 1956). In these fishes, the administration of estrogen caused the liver cells of the male to be histologically similar to those of the female, though, in the medaka, the treatment of males in their sexually inactive period could not induce any effects on the liver cells. From these findings, together with the results of the present study, it may be concluded that female gonadal hormones, which may be secreted actively during the breeding season, account for bringing about sexual differences in the liver cells of teleost fishes.

In the present study, some of the cytological changes of the liver cell in ethinylestradiol-treated fish are regarded as pathological ones. This study presents an expansion in size, vacuolizations and decrease in density of the matrices in the mitochondria, or the appearance of a vesicular endoplasmic reticulum and a frequent occurrence of dense bodies with membraneous inclusion in the affected liver cells. This may be due to the extra-physiological dose of the estrogen mentioned in the present study. Nevertheless, the features found in the liver cells influenced by the estrogen were closely similar to those found in the cells of mature females. It seems to be safe to regard the remarkable changes in glycogen deposits and in the endoplasmic reticulum as a strengthened expression of female characters of the liver cells.

It has been reported that, in a frog *Xenopus laevis*, estrogen induces hepatic synthesis of a serum lipophosphoprotein which is selectively uptaken by the ovary and is transformed into yolk platelet proteins (Wallace & Jared, 1969). The facts that both immature and ovariectomized goldfish came to have a large amount of glycogen deposit in the liver, and that the glycogen deposit was lessened in amount in mature fish or after the treatment of the ovariectomized fish with estrogen, suggest a possibility that, also in the fish, ovarian hormones may play some role in releasing the material stored in the liver and transferring it to the maturing

ovary. Future studies are needed to clarify the role of the liver in view of its connection to the maturation of the gonad.

Summary

In sexually mature goldfish, sexual differences were recognized in the structure of the liver cells. The liver cells of mature females were smaller in size and with roundish nucleus in comparison with those of mature males which were similar in structure to those of immature fish of both sexes. Electron microscopically, the female liver cells had much less glycogen deposit and a much more developed endoplasmic reticulum in the cytoplasm. Ovariectomy of mature females caused alterations of the liver cells from the type of the female to that of the male. The administration of ethinylestradiol to ovariectomized or to immature fish of both sexes induced a hypertrophy of the nucleus, depletion of glycogen and a prominent increase in amount of endoplasmic reticulum, strongly facilitating the appearance of the cells of the female type in the treated fish. On the contrary, methyltestosterone induced no changes in the liver cells of immature fish. It was confirmed that the sexual differences found in the liver cells of mature goldfish were caused by a certain action of ovarian hormones on the liver cells.

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Explanation of Plates

PLATE I

All figures are photomicrographs from sections through the liver of the goldfish, *Carassius auratus*, magnified at ca. 1,000 times

Fig. 1. Liver of a normal female in a sexually inactive period

Fig. 2. Liver of a normal female in the breeding season

Fig. 3. Liver of a normal male in the breeding season

Fig. 4. Liver of an ovariectomized female 45 days after operation

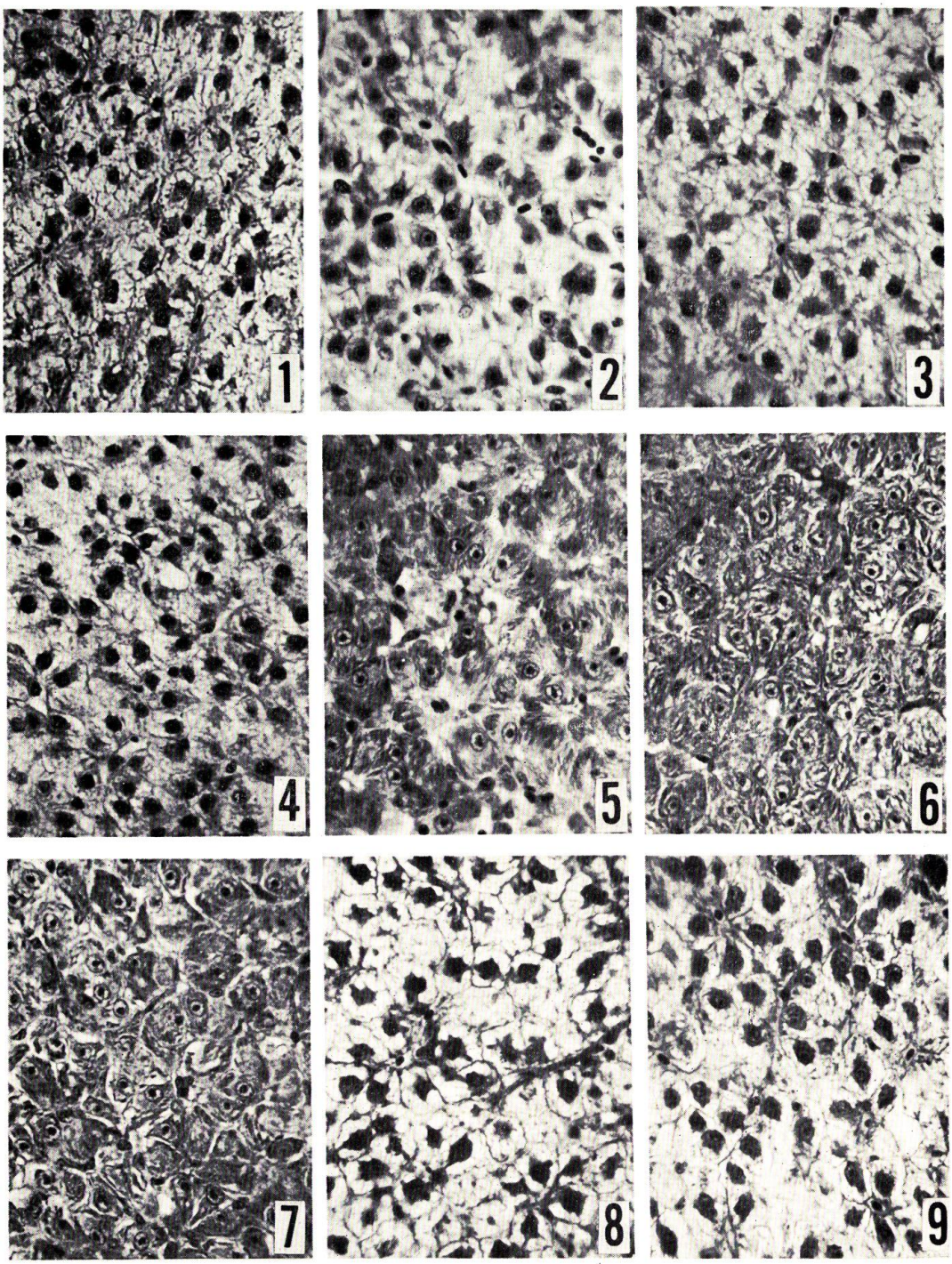
Fig. 5. Liver of an ovariectomized female treated with ethinylestradiol

Fig. 6. Liver of an immature female treated with ethinylestradiol

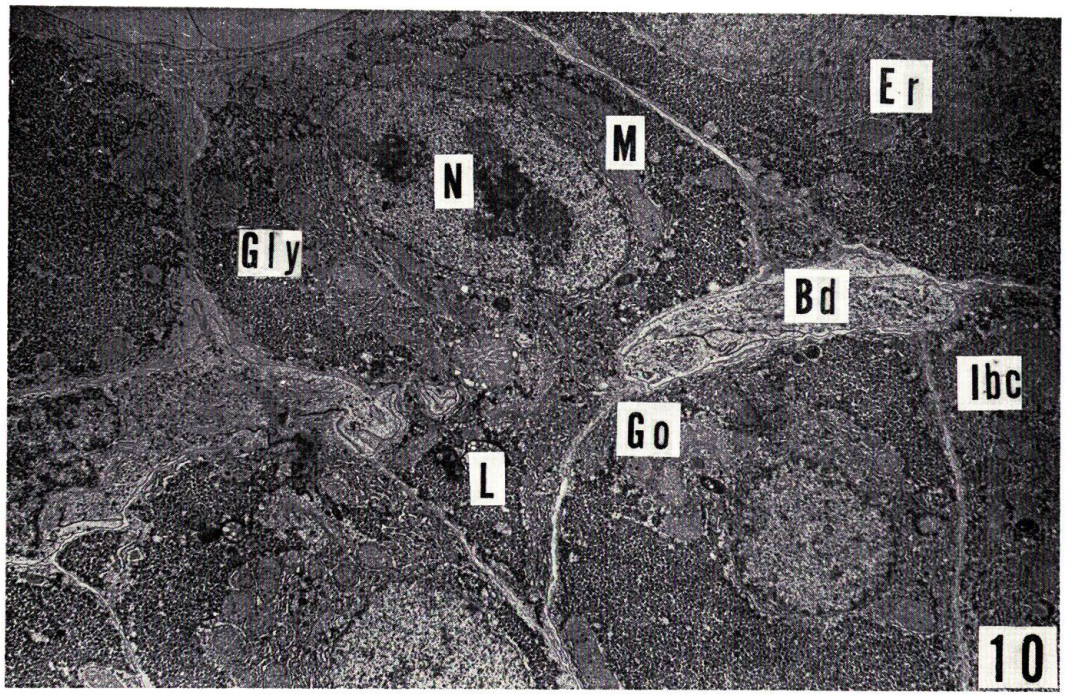
Fig. 7. Liver of an immature male treated with ethinylestradiol

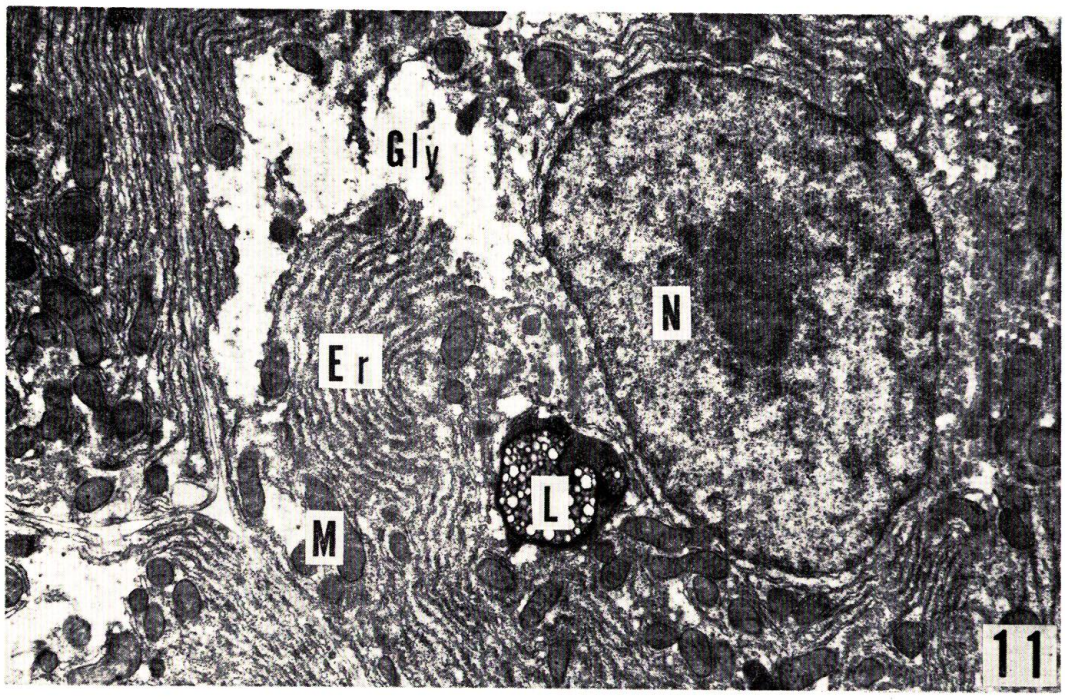
Fig. 8. Liver of an immature female treated with methyltestosterone

Fig. 9. Liver of an immature male treated with methyltestosterone



K. ISHII & K. YAMAMOTO: Sexual differences of the goldfish liver cells





K. ISHII & K. YAMAMOTO: Sexual differences of the goldfish liver cells

PLATE II

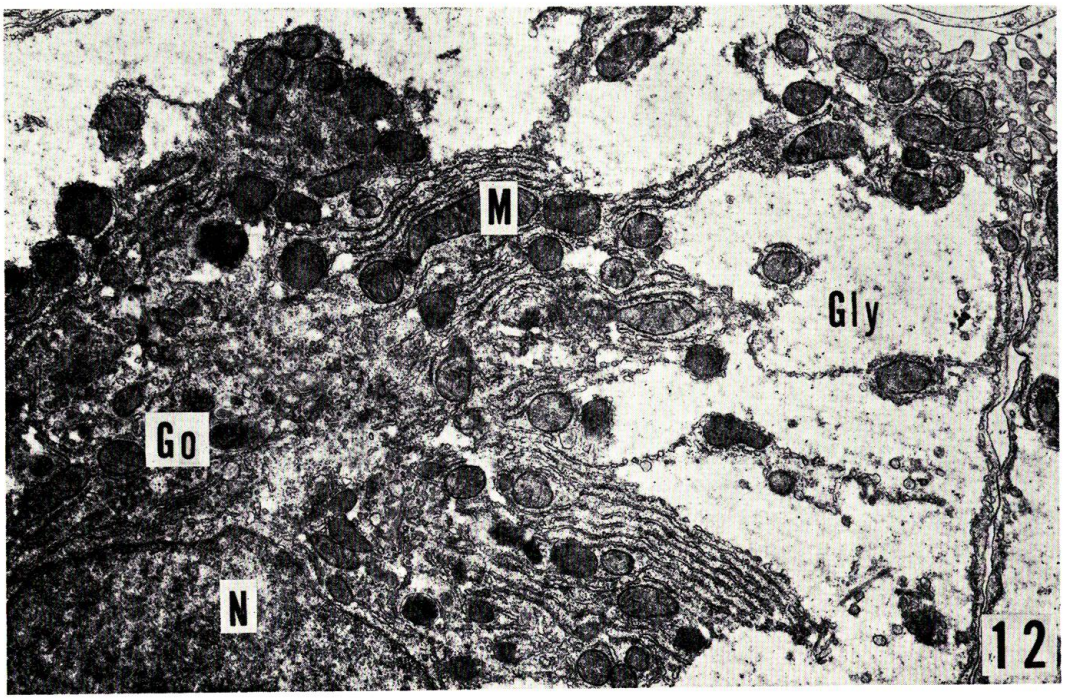
Fig. 10. Electron micrograph of liver parenchymal cells in a normal female goldfish in a sexually inactive period. Various cytoplasmic organelles are seen concentrated in the perinuclear region, while glycogen granules occupy the periphery of the cell. *Bd*, bile duct; *Er*, endoplasmic reticulum; *Gly*, glycogen deposit; *Go*, Golgi complex; *Ibc*, intracellular bile capillary; *L*, lysosome; *M*, mitochondrion; *N*, nucleus, ×6,600

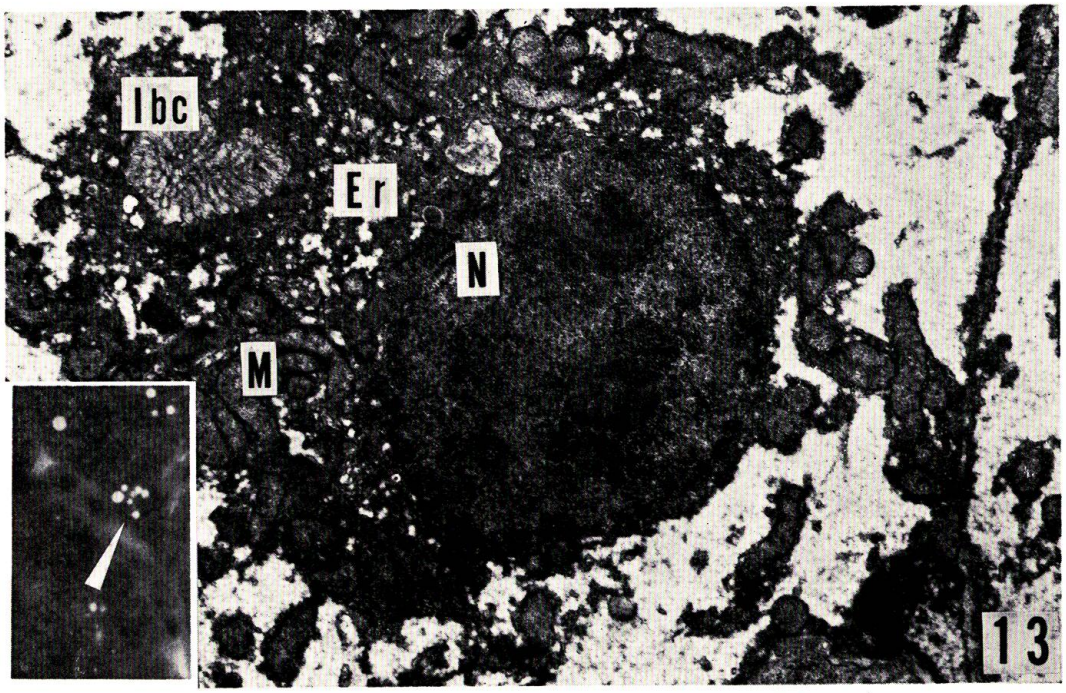
Fig. 11. Electron micrograph of the liver cell in a normal female goldfish in the breeding season, showing extensive development of the rough endoplasmic reticulum in the cytoplasm and a poor deposit of glycogen granules. *Er*, endoplasmic reticulum; *Gly*, glycogen deposit; *M*, mitochondrion; *L*, lysosome; *N*, nucleus, ×10,500

PLATE III

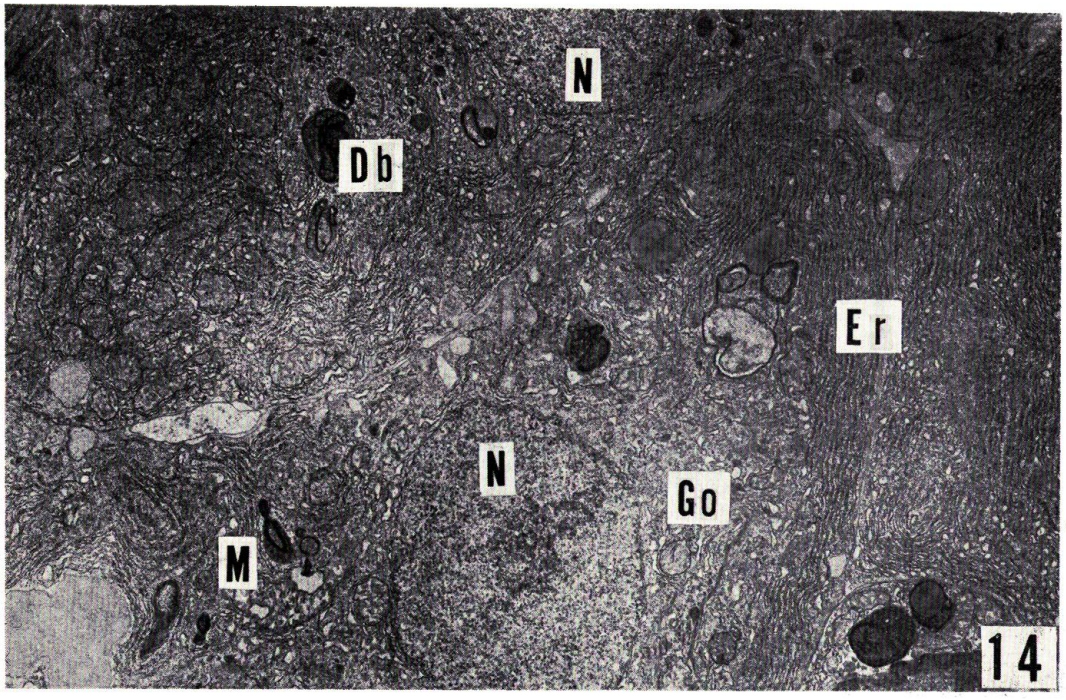
Fig. 12. Electron micrograph of the liver cell in a normal male goldfish in the breeding season, revealing much glycogen deposit in the periphery of the cell. *Gly*, glycogen deposit; *Go*, Golgi complex, *M*, mitochondrion; *N*, nucleus, ×10,500

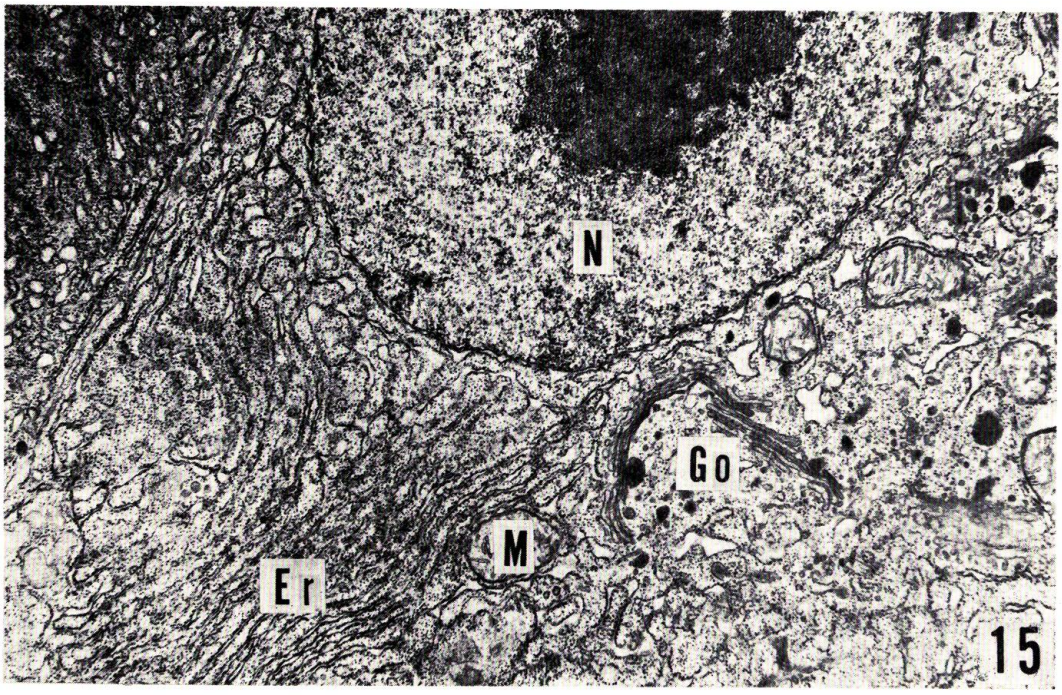
Fig. 13. Electron micrograph of the cell of an ovariectomized goldfish, 45 days after ovariectomy. The endoplasmic reticulum does not show prominent development of the stacks of flattened cisternae. *Er*, endoplasmic reticulum; *Ibc*, intracellular bile capillary; *M*, mitochondrion; *N*, nucleus. ×10,500. Inset. A light microscopic picture of a section of the liver cell 45 days after ovariectomy. Some of the cells contain fat droplets (arrow) in the cytoplasm. OsO_4, Richardson's stain, ×1,000





K. ISHII & K. YAMAMOTO: Sexual differences of the goldfish liver cells





K. ISHII & K. YAMAMOTO: Sexual differences of the goldfish liver cells

PLATE IV

Figs. 14 and 15. Electron micrographs of the liver cells of ovariectomized goldfish treated with ethinylestradiol. Note the depletion of glycogen granules and a prominent development of the endoplasmic reticulum in the cells. *Db*, dense body; *Er*, endoplasmic reticulum; *Go*, Golgi complex; *M*, mitochondrion; *N*, nucleus. Fig. 14, ×6,600; Fig. 15, × 13,200