Effects of Vitamin A Administration upon Ito’s Fat-Storing Cells of the Liver in the Carp

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Summary. In the normal carp livers, Ito cells (fat-storing cells) could be identified with light and electron microscope within the hepatic sinusoidal wall and actually in the Disse’s space, but they were almost lacking lipid droplets in the cytoplasm (empty Ito cells) in contrast to the majority of vertebrate species.

After administration of large doses of vitamin A, fat droplets appeared in hypertrophic cytoplasm of enlarged Ito cells, and their size and number increased roughly in proportion to the amounts of vitamin A administered. This evidence was thought to demonstrate that the administration of large doses of vitamin A could convert empty Ito cells into lipid containing ones and further suggested that excess vitamin A administered might be stored in newly prepared lipid droplets of the Ito cells of the liver. In experimentally hypervitaminotic carp, hypertrophic Ito cells showed proliferation of fine filaments in addition to the accumulation of lipid droplets. The correlation between perisinusoidal fibrogenesis in the hepatic lobule and the proliferation of cytoplasmic filaments in the Ito cells was discussed.

The fat-storing cell was discovered in normal human livers, and for the first time reported by ITO (1951) and then described in detail by ITO and NEMOTO (1952). The fat droplet containing cells were shown to occur within the hepatic sinusoidal wall and to differ from Kupffer and sinusoidal endothelial lining cells in their location and by the lack of phagocytic activity (SATSUKI, 1952). Light microscope studies by Ito and his coworkers have demonstrated that the liver of mammals, birds, reptiles, amphibia and even fishes possessed these cells, although certain dimensional and numerical species differences in their fat droplets were noticed (ITO et al., 1952; Ito, 1973).

The electron microscopic evidence that the Ito cell (fat-storing cell) is located in the perisinusoidal space of Disse was established first by YAMAGISHI (1959) and then by many others (ITO, 1973).

Recently, the remarkable increase of fat droplets in the Ito cell after administration of vitamin A has been reported by KOBAYASHI et al. (1970, 1971, 1973), WATARI and TORISAWA (1970), WAKE (1971, 1974), HIROSAWA and YAMADA (1973), YAMAMOTO (1975), KUSUMOTO and FUJITA (1977) and others. Thus, as first proposed by NAKANE (1963) and BRONFENMAJER et al. (1965), the vitamin A-storing cells of the liver have come to be identified with the Ito cells. Some of the above authors have actually revealed the presence of vitamin A in lipid droplets of Ito cells of hypervitaminotic animals by means of fluorescence microscopy or radioautography at the electron microscope level (HIROSAWA and YAMADA, 1973; YAMADA and HIROSAWA, 1976).

Several years ago, ITO, WATANABE and TAKAHASHI (1962) light microscopically investigated the Ito cells in 48 kinds of bony fishes, kinds of cartilaginous fishes and...
lampreys (Cyclostoma) and showed that the Ito cells of the liver in almost all kinds of fishes examined (183 examples in total) contained lipid droplets blackened with osmic acid, except in the 3 kinds of fresh water bony fishes, i.e., carp, ayus and killifishes. Especially, the livers of adult carp (3 years) were collected monthly throughout one year to examine the fat droplet contents of the Ito cells, but no droplets were detected in them.

The aim of this paper is to answer the question whether physiologically empty Ito cells of the carp can transform into fat droplet-containing cells in the experimentally hypervitaminotic state.

**Material and Methods**

Twenty carp (Cyprinus carpio Linne) were divided into three groups. In the first, seven carp were intraperitoneally given an aqueous solution of vitamin A palmitate (Chocola A, Eisai Co., Ltd. Japan) in a dose of 100,000 I.U./kg once a day for five days. In the second, seven carp were given 100,000 I.U./kg once a day for ten days and six carp of the third group were used as controls. The animals in groups 1 and 2 were killed 24 hrs after the last injection. For light microscopy, blocks of liver tissue were fixed, some in Levi’s fluid, others in Zenker-formol, and still others in Bouin and all embedded in paraffin. Sections were stained with azocarmine-aniline blue, hematoxylin-eosin, and PAS-hematoxylin. For electron microscopy, small pieces of tissue were fixed in a 5% glutaraldehyde, buffered with cacodylate for 2 hrs and postfixed with a 1% osmic acid solution with phosphate buffer (Millonig) for another hour. The pieces were dehydrated in a series of graded ethanol concentrations and embedded in Epon. Sections cut on a Porter-Blum MT-II ultramicrotome were stained with uranyl acetate and lead citrate and examined with JEM 100U and 100C electron microscopes.

**Observations**

1. **Light microscopy**

   **Control carp**

   In control carp, Ito cells do not contain any fat droplet in their cytoplasm, but they can be distinguished from other type cells, namely, hepatocytes, Kupffer and endothelial cells. Under high power view of the preparations fixed with Levi’s fluid and stained with azocarmine-aniline blue, Ito cells devoid of fat droplets (empty Ito cells) are located within the sinusoidal wall mainly in hollows induced between hepatocytes and are separated from the sinusoidal lumen by a blue stained line corresponding to reticular fiber (Fig. 1). These cells are confirmed as empty Ito cells.

   **Fig. 1.** Light micrograph of a control carp liver. An Ito’s fat-storing cell (FC) is found in a hollow between hepatocytes. E endothelial cell, HC hepatocytes, KP Kupffer cell. Levi, Azan. \( \times 1,260 \)

   **Fig. 2.** Light micrograph of vitamin A injected carp liver (Group 1). An Ito cell (FC) contains several fat droplets blackened by osmic acid. E endothelial cell, HC hepatocytes, R erythrocyte. Levi, Azan. \( \times 1,260 \)

   **Fig. 3.** Light micrograph of carp liver after injection of larger doses of vitamin A (Group 2). Two Ito cells (FC) having numerous fat droplets of variable sizes in their cytoplasm are seen within the sinusoidal wall. Lipid droplets scattered in hepatocytes (HC) are also increased in number. E endothelial cell, KP Kupffer cell. Levi, Azan. \( \times 1,260 \)
Fig. 1–3. Legends in opposite page.
also by electron microscopy (vide infra). The endothelial cell, directly exposed to the sinusoidal lumen has an elongated oval nucleus and protrudes, along the sinusoidal surface long cytoplasmic sheets from both sides of the nucleus. The Kupffer cell is located within the sinusoidal lumen and has a large oval or round nucleus, and a wide cytoplasmic layer around the nucleus contains lysosomal granules.

**Vitamin A injected groups**

In the liver of carp which received large doses of vitamin A for five days (Group 1), fat droplets appear in the cytoplasm of almost all Ito cells distributed throughout hepatic lobules. Medium-sized fat droplets, blackened with osmic acid, are packed in the cytoplasm (Fig. 2); their size and number may vary from cell to cell. In those given larger doses of vitamin A (Group 2), Ito cells contain more numerous fat droplets and become conspicuously enlarged. Lipid droplets around the nucleus increase in number and size, but among strikingly large droplets there appear smaller ones and some of the lipid droplets are frequently sporadically distributed in cytoplasmic processes sent out from the cell body along the sinusoidal surface (Fig. 3). Lipid droplets contained in hepatocytes are slightly increased in number, while they are few in Kupffer and endothelial cells. In both experimentally hyper-vitaminotic cases empty Ito cells have almost disappeared.

2. **Electron microscopy**

**Control carp**

In electron micrographs of the normal liver, Ito cells are found in the perisinusoidal space of Disse. Thus, Ito cells are separated from the sinusoid by the endothelial lining. As revealed by electron microscopy, Ito cells of the control carp demonstrate no fat droplets. Even in the state lacking in fat droplets, these Ito cells

![Fig. 4. Electron micrograph of a control carp liver. An empty Ito cell (FC) devoid of fat droplets is seen between the sinusoidal endothelium (E) and hepatic parenchymal cells (HC). An oval nucleus is surrounded by a narrow cytoplasmic layer. S sinusoid. ×12,000](image-url)
have some characteristic features besides their location above mentioned: they protrude long cytoplasmic sheaths beneath the endothelial lining, possess numerous slender microvillii at the cell surface, have dilated cisterns of granular endoplasmic reticulum and moderately dilated perinuclear space, and contain small amounts of fine filaments in the cytoplasm of control carp. These empty Ito cells are small in size, and give a low nucleo-plasmic ratio, so that a small oval nucleus with a nucleolus is invested by a narrow cytoplasmic layer, which contains small oval mitochondria, granular endoplasmic reticulum, free ribosomes and the Golgi apparatus consisting of stacks of short cisterns (Golgi lamellae) and some associated vesicles (Fig. 4). In exceptional cases a few small fat-droplets (vacuoles) are revealed in the cytoplasm of the Ito cell.

**Vitamin A injected groups**

In the carp given vitamin A (Group 1), numerous fat droplets (vacuoles) appear as revealed by light microscopy, in the cytoplasm of remarkably enlarged Ito cells (Fig. 5). The size of these fat droplets varies widely in one and the same cell and they pack the hypertrophic cytoplasm to make frequently large depressions on the nuclear envelope. In contrast to the cytoplasm, the nucleus is not conspicuously hypertrophic. Among these fat droplets there remain here and there considerably

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**Fig. 5.** Electron micrograph of vitamin A injected carp liver (Group 1). A considerably hypertrophic Ito cell (FC) having several fat droplets (vacuoles) (L) is found between the sinusoidal endothelium (E) and hepatocytes (HC) protruding numerous microvilli (MV). C collagen or reticular fiber closely abutting on the surface of the Ito cell, f bundle of filaments along the cell surface, G Golgi apparatus, R erythrocyte, S sinusoid. ×11,000
wide areas of cytoplasm which contain somewhat dilated cisterns of granular endoplasmic reticulum of irregular form filled with a dense material, free ribosomes, vacuoles containing moderately electron dense material (lysosomes) and often fibrillar structures (Fig. 5). Mitochondria are not conspicuous, and on one side of the nucleus a prominent Golgi apparatus is identified, composed mainly of stacks of flattened cisterns (lamellae) and numerous small vesicles containing electron dense material.

In the carp given larger doses of vitamin A (Group 2), the hypertrophy of the cytoplasm of Ito cells becomes much more striking, and many of them, packed by numerous lipid droplets, are scattered in the Disse’s space of hepatic lobules. Fat droplets in Ito cells are increased in size and number, and some of them, closely packed in cytoplasm, fuse with each other into larger ones of irregular shapes (Fig. 6). Extraordinarily large spherical lipid droplets which are encountered in the Ito cells in these cases have probably been produced in this way. The narrow cytoplasmic layer among lipid droplets contains mitochondria, lysosomes, free ribosomes and conspicuously expanded cisterns of rough endoplasmic reticulum filled with an electron dense material. In hypervitaminotic carp, the hypertrophic cytoplasm of the Ito cells exhibits fine filaments making bundles along the cell surface (Fig. 5, 6), between lipid droplets as well as between cell organelles and around the nucleus (Fig. 7). The amount and distribution of filament bundles vary from cell to cell; an
Ito cell depicted in Figure 7 contains a large amount of filaments, forming thick bundles. Hypertrophy of the nucleus of the Ito cell is not conspicuous, but one may frequently find depressions induced by lipid droplets on the nuclear envelope.

**Discussion**

It has been long known that empty Ito cells are present especially in the central zone of the hepatic lobules of vertebrates (ITO and NEMOTO, 1952; KANO, 1952; SUNAGA, 1954; IMAI, 1967; ITO and SHIBASAKI, 1968; OKUDA et al., 1968; ITO, 1973; YAMAMOTO, 1975). These cells have been considered as reserve cells which retain a fat-storing capacity. Although they lack lipid droplets, they can be distinguished by morphological features characteristic of the Ito cell. The existence of vertebrate species in which Ito cells in the liver are entirely lacking lipid droplets in the physiological condition is thought to be exceptional. In their light microscopic study on livers in a vast variety of fishes, ITO, WATANABE and TAKAHASHI (1962) have discovered 3 kinds of fresh water bony fishes, i.e., carp, ayus and killifish whose Ito cells were completely lacking in lipid droplets. To corroborate this result, carp were collected monthly throughout one year for the examination of Ito cells, since it has been believed that fresh water fishes may undergo seasonal changes in Ito cells and hepatocytes however, no fat droplets were ever found in the cytoplasm of Ito cells throughout the year and any other seasonal changes could not be detected in the cells by light microscopy (ITO et al., 1962).

In the present study, both light and electron microscopy have confirmed that
the majority of Ito cells of the carp examined are lacking in lipid droplets. Thus, it is characteristic of carp that the Ito cells of the liver are more or less completely devoid of lipid droplets. Recently, however, it has been reported by a number of authors that administration of vitamin A in rats results in an increase in number and size of fat droplets in the Ito cell (Kobayashi and Takahashi, 1970, 1971; Watari and Torisawa, 1970; Kobayashi, Takahashi and Shibasaki 1973; Wake, 1971, 1974; Hiroawa and Yamada 1973; Yamamoto, 1975). It has been proposed that Ito cells accumulate lipid droplets in order to store fat soluble excess vitamin A (Kobayashi and Takahashi, 1971). If this is also the case with carp, it may be expected that the administration of a large dose of vitamin A would induce the appearance of lipid droplets in their empty Ito cells to store excess vitamin A.

The present study has actually revealed that administrations of large doses of vitamin A transformed the empty Ito cells into lipid containing ones. The cells underwent more of less hypertrophy and the increase in number and size of fat droplets was in proportion to the total amount of vitamin A administrated. These changes in the Ito cells likely reflect the rise in blood level of vitamin A induced by this experiment. This result indicates that in certain species of fresh water bony fishes lipid droplets of the Ito cells may be demonstrable, only in hypervitaminotic states. Several years ago, Schmidt (1956) examined normal carp livers and reported that cells corresponding to the Ito cells failed to be demonstrated. This negative result is probably due to his failure to recognize empty Ito cells in normal carp.

It has recently been pointed out that in experimentally hypervitaminotic animals, vitamin A-storing cells can be demonstrated in various organs and areas (connective tissues) other than the liver (Hiroawa and Yamada, 1974, 1977; Yamada and Hiroawa, 1976; Kusumoto and Fujita, 1977). These authors have disclosed lipid droplet-containing cells exhibiting vitamin A reaction in connective tissues of the lung, stomach, small intestine, adrenal gland, spleen, lymph nodes, thymus and bone marrow. In the present study, extrahepatic fat-storing cells of the carp were not examined, though it seems an interesting problem to be investigated.

The view that the Ito cells should be responsible for intralobular fibrogenesis in the Disse's space has been proposed by many authors (Wood, 1963; Schnack et al., 1966, 1967; Wewalka et al., 1966; Stockinger, 1969; McGee and Patrik, 1967, 1972; Imai, 1967; Kondo, 1967; Ito and Shibasaki, 1968; Poper and Udenfriend, 1970, Kawanami, 1973; Ito, 1973; Yamamoto, 1975, etc.). The present study has often revealed, in the cytoplasm of Ito cells from hypervitaminotic carp (Group 1 and 2), the proliferation of fine filament bundles, their profiles of variable thickness, being distributed along the surface of the Ito cells, around the nucleus and between cell organelles. In addition, more or less distended cisterns of the rough endoplasmic reticulum containing an electron dense material have been observed, suggesting an accelerated fibrogenesis in the Ito cell. Thus, it has been proved that hypervitaminosis not only promotes the accumulation of lipid droplets but also filament formation in the Ito cell. However, the question whether lipid storage in the Ito cell and fibrogenesis in the Disse's space might actually be correlated is unknown. It is worthy to mention in this connection that patients who received prolonged administration of vitamin A occasionally manifest hepatic fibrosis. Some authors suggested that the vitamin A may have stimulated fibrogenesis in the liver lobules in those patients (Muenter et al., 1971; Hruban et al., 1971; Russell et al., 1974).
Ehrlich et al. (1973) and Lee et al. (1973) reported accelerated fibrogenesis also in the skin after administrations of excess doses of vitamin A.

**References**


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