An Ultrastructural-Cytochemical Study on the Proliferation of Smooth Endoplasmic Reticulum Induced by Chlorobiphenyls (PCB) in the Guinea Pig Liver Cells

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Summary. The ultrastructural changes in the liver cells of guinea pigs induced by the oral administration of PCB were studied by electron microscopy; also electron-microscopic cytochemistry for glucose-6-phosphatase (G-6-Pase) activity was applied. Proliferation of smooth endoplasmic reticulum (sER) was the most prominent change observed in the liver cells, which remained as long as 90 days after the final administration.

G-6-Pase activity was ultracytochemically demonstrated not only in the rough and smooth endoplasmic reticulum and the nuclear envelope in the liver cells of normal controls, but also in the proliferated sER in the liver cells of PCB-treated animals.

The present investigation revealed that PCB stored in the animal body induced the proliferation of sER in the liver cells for a long time after the cessation of the treatment, and that sER in the liver cells, normally existing or proliferated, always showed positive activity of G-6-Pase.

Recently, several electron microscope studies have been reported on the ultrastructural changes of the liver caused by the administration of chlorobiphenyls (PCB), after the outbreak of PCB poisoning that involved a great many people in Western Japan in 1968, who used rice bran oil contaminated with PCB (Kanechlor 400) (Yamamoto, 1970; Nishizumi, 1970; Kimbrough et al., 1972). The result common to these investigations was that PCB induced a remarkable proliferation of smooth endoplasmic reticulum (sER) in the liver cells of experimental animals. The proliferation of sER of the liver cells was also observed in the liver biopsied from a patient suffering from PCB poisoning (Yamamoto, 1970).

It has been shown from biochemical analyses that PCB once taken up into an animal body remains in various tissues for a long time (Yoshimura et al., 1971).

Thus the present study was carried out to clarify by means of electron microscopy how long the effect of PCB remains in the liver cells in animals after the cessation of PCB administration. In addition, the ultracytochemical activity of glucose-6-phosphatase (G-6-Pase), which is a microsomal membrane-bound enzyme (Ernster et al., 1962) was investigated to know if the proliferated smooth membranes of liver cells always retain the same enzymatic characterization as that of normal smooth membranes.

Materials and Methods

Male guinea pigs (about 400g) were used for the present study. The animals received daily oral administration of 5mg of Kanechlor 400 (PCB) per kg body weight
for 3 days successively (about 0.2ml of 1% PCB in olive oil was given per day). The controls received the same amount of olive oil without PCB. Another control received the same diet with no oil or PCB. All animals were fed a standard commercial laboratory animal diet and were starved overnight just before being killed by decapitation. Of all the experimental animals, the guinea pigs were killed two at a time on the 1st, 7th, 14th, 21st, 28th, 46th, and 90th day after the final administration of PCB.

For electron microscopy, small pieces of liver tissue were removed from the experimental animals and the controls. After being cut into small bits, the specimens were fixed in an ice cold 1% osmium tetroxide buffered with 0.1M s-collidine at pH 7.4 for 2hrs. After dehydration in a graded series of ethanol solutions, starting with 50%, the materials were embedded in epoxy resin. Both thick and thin sections were made with glass knives on a Porter-Blum microtome. The former was stained with toluidine blue and examined under a light microscope. The latter sections were stained with lead tartrate solution and examined in a Hitachi HS-7D electron microscope.

For the cytochemical studies of G–6–Pase, some of the pieces of the liver were fixed in an ice cold 2% glutaraldehyde buffered with 0.1M cacodylate for 40 min, then the tissues were rinsed in 0.1M cacodylate buffer overnight. Frozen sections of approximately 40μm thick were made on a Spencer’s Cryocut microtome. The sections were incubated in the Wachstein-Meisel medium (WACHSTEIN and MEISEL, 1956) for 20 min at room temperature. As the controls for cytochemical studies, the sections were post-fixed in an ice cold 1% osmium tetroxide buffered with 0.1M s-collidine at pH 7.4 for 1 and a half hr. Following fixation, the sections were dehydrated and embedded in the same way as stated above. However, the thin sections were studied either unstained or stained with uranyl acetate and/or lead tartrate.

Observations

A. Ultrastructural Changes

1. Normal controls

In the animals fasted overnight, there was a marked depletion of glycogen particles in the cytoplasm of liver cells (Fig. 1). The smooth endoplasmic reticulum (sER) was clearly visualized. The sER was distributed at random throughout the cytoplasm. It appeared to consist of vesicles and a meshwork of tubules with anastomosis. The rough endoplasmic reticulum (rER) revealed their thin flattened cisternae studded with ribosomes and was distributed widely in the cytoplasm. A direct continuity between rER and sER was encountered occasionally. The Golgi complex was usually located near the bile capillary, but it also occurred near the nucleus. Mitochondria of oval or ellipsoidal shape were scattered in the cytoplasm. Lysosomes, round in outline and polymorphic in content, occurred infrequently. Microbodies were observed as granular components, limited by a single membrane, with homogeneous matrix, and were smaller in size and fewer in number than mitochondria. There was little accumulation of lipid droplets in the cytoplasm. Free ribosomes were seen throughout the cytoplasm.
2. Olive oil treated animals

Liver cells of the animals, which had received daily administration of 0.2ml of olive oil for 3 successive days exhibited no marked difference in fine structure from those of normal controls. Both rER and sER were almost the same in shape, distribution and amount as those of the normal controls. No changes were seen in other organelles. However, in some of the liver cells, a few lipid droplets could be found in the cytoplasm.

3. PCB treated animals

On the 1st day after the cessation of the treatment, liver cells exhibited the characteristic change of a marked proliferation of sER. A remarkable increase in the amount of sER appeared in the liver cells throughout the lobule (Fig. 2). The proliferation of the sER was so extensive that smooth-surfaced membranous profiles occupied almost all the cytoplasm and put other organelles aside. The rER was well developed and it was frequently found to have clear continuity with the sER. The latter was observed generally as tubular constituents with branching and continuity, and it seemed to grow up from the ends of flattened cisternae of the rER, when proliferated. Except for the remarkable increase of the sER, other cell organelles
Fig. 2. Liver cells on the 1st day after the cessation of PCB treatment. Proliferation of sER is seen throughout the cytoplasm. Sites of its continuity with rER are frequent. Free ribosomes are scattered in the hyaloplasm. ×10,000

Fig. 3. Liver cells on the 7th day after cessation of the treatment. Proliferation of sER is more marked. Smooth-surfaced membranous profiles are more closely meshed. Anastomosing tubules of the proliferated sER are observed throughout the cytoplasm. The continuity between the rER and sER is seen. ×10,000
such as Golgi complex, mitochondria, lysosomes and microbodies did not show any alterations in shape, size and number. A few lipid droplets occurred in some of the liver cells.

On the 7th day after cessation of the treatment, the fine structural changes of the liver cells were most conspicuous. The proliferation of the sER was more marked than on the 1st day after cessation of the treatment (Fig. 3). So the smooth-surfaced membranous profiles appeared to be more closely meshed. The rER was also well developed. There were many points where the rER and sER were in continuity with each other. The Golgi complex did not show any alterations. Mitochondria and microbodies exhibited little alteration in size, shape and number. Lysosomes appeared to be slightly increased in number. Some of them contained myelin figures. Liver cells containing a few lipid droplets were frequently encountered.

On the 14th day after cessation of the treatment, the proliferation of the sER was still marked, though not so conspicuous as compared with that seen in the animals above mentioned. There were no changes in number, size and shape of mitochondria and microbodies. The Golgi complex showed no changes. Lysosomes were not increased in number. Liver cells containing a few lipid droplets were not frequently seen.

On the 21st day after cessation of the treatment, the proliferation of the sER was still recognized, and its extent was almost the same as seen on the 14th day after the final administration (Fig. 4). The other cell organelles did not show any alterations.

On the 28th day after cessation of the treatment, the proliferation of the sER was persistent. The ultrastructural changes, including proliferation of the sER, were
almost the same as on the 21st day after the final administration.

On the 46th day after cessation of the treatment, the sER was still proliferated, though the extent was not so marked as compared with that seen on the 28th day after the final administration (Fig. 5). The proliferated sER was not so closely meshed, and smooth-surfaced membranous profiles did not occupy the cytoplasm so widely as those did on earlier days after the final administration. Other cell organelles did not show any alterations. A few lipid droplets were seen in many cells.

Fig. 5. Liver cells on the 46th day after cessation of the treatment. Proliferation of the sER is recognized, but is not so marked in extent. ×9,000

On the 90th day after cessation of the treatment, the proliferation of the sER was still found. The membranous profiles of the sER were not so concentrated, nor so closely meshed as those were in the liver cells examined earlier after the final administration. No remarkable changes were seen in other organelles. A few lipid droplets were frequently seen in the liver cell cytoplasm.

B. Cytochemistry: Localization of Glucose-6-Phosphatase

1. Normal controls

Localization of G-6-Pase activity was recognized where lead reaction product was observed on electron micrographs.

Lead reaction product was observed in both rough and smooth endoplasmic reticulum, and also in the nuclear envelope. No reaction product was observed in Golgi complex, mitochondria, microbodies, lysosomes and lipid droplets, nor on plasma membranes (Fig. 6). The reaction product filled the cavity of the endoplasmic
Fig. 6. Liver cells of normal control, showing the localization of G-6-Pase activity. Lead reaction product which indicates the localization of G-6-Pase activity is seen in the endoplasmic reticulum and the nuclear envelope. No reaction product is seen on the nucleus, mitochondria, lysosomes, Golgi complex, and plasma membranes. ×10,800

Fig. 7. A liver cell of a normal control, showing the localization of G-6-Pase activity. The reaction product is seen in the cisternal side of the endoplasmic reticulum and the nuclear envelope, but not in the cytoplasmic matrix. ×13,600
Fig. 8. Liver cells on the 1st day after the cessation of the treatment, showing the localization of G-6-Pase. The reaction product is seen in the nuclear envelope and the endoplasmic reticulum including the proliferated sER. ×10,000

Fig. 9. Liver cells on the 7th day after the final administration, showing the localization of G-6-Pase activity. Lead reaction product is seen in the markedly proliferated sER. ×18,000
reticulum, but no reaction product was seen in the cytoplasmic matrix (Fig. 7). G-6-Pase activity was positive only in the liver cells. The tissues incubated in the medium lacking glucose-6-phosphate showed no reaction product in the liver cells.

2. PCB-treated animals

The fine structural localization of G-6-Pase activity in the PCB-treated animals was essentially similar to that observed in the normal controls. The reaction product was observed in the nuclear envelope, r- and sER including the proliferated sER (Fig. 8). Figure 9 shows the positive reaction product in the markedly proliferated sER observed in the liver cells on the 7th day after the last administration of PCB. G-6-Pase activity was positive in the proliferated sER observed on the 14th, 21st, 46th, and 90th day after the final administration of PCB (Fig. 10). G-6-Pase activity was positive only in the liver cells. The tissues incubated in the medium lacking glucose-6-phosphate showed no reaction product in the liver cells.

Discussion

It has been well known that the sER is more or less markedly proliferated in the liver cells after the administration of a wide variety of lipid-soluble chemical agents such as 3'-MeDAB (Porter and Bruni, 1959), 2-MeDAB (LaFontaine and Allard, 1964), phenobarbital (Remmer and Merker, 1965; Orrenius, Ericsson and Ernster, 1965; Jones and Fawcett, 1966), tolbutamide (Remmer and Merker, 1965), chlordane (Fouts and Rogers, 1965), DDT (Ortega, 1966), progesterone (Emans and Jones, 1966), thiohydantoin derivative (Herdson et al., 1967). Among these chemical agents...
Phenobarbital is the most famous inducer of sER proliferation in the liver cells. Correlated with this proliferation, there is an enhancement of the activity of microsomal drug-metabolizing enzymes. These changes are interpreted as adaptations to increase the efficiency of the liver in the elimination of these drugs. One of the functions of the sER of the liver cells is considered to be the enzymatic degradation and elimination of lipid-soluble drugs (Remmer and Merker, 1965; Jones and Fawcett, 1966). Chlorobiphenyls (PCB) caused a proliferation of sER in the liver cells of rabbits and guinea pigs (Yamamoto, 1970), rats (Tomio, 1969; Yamamoto, 1970; Kimbrough et al., 1972), mice and monkeys (Nishizumi, 1970). PCB also induced the liver microsomal drug-metabolizing enzymes in guinea pigs (Yamamoto, 1971) and in rats (Fujita et al., 1971). Proliferation of sER was also observed in the liver cells of a patient suffering from PCB poisoning (Yamamoto, 1970).

On the mechanism of the induction of sER proliferation in the liver cells by chemical agents as above mentioned, there have been many reports from morphological, biochemical and cytochemical viewpoints. Morphologically, as was reported in the phenobarbital-induced sER proliferation (Orrenius and Ericsson, 1966a), the continuity between the rER and sER was frequently encountered in the liver cells of the guinea pigs treated with PCB. This may suggest that the proliferated smooth membranes are newly formed by the rER membranes. Biochemically, phenobarbital treatment caused an increase in liver microsomal protein, RNA, and phospholipid (Orrenius, Ericsson and Ernster, 1965). Simultaneous administration of phenobarbital with actinomycin D which blocks the DNA-dependent synthesis of messenger-RNA, or with puromycin which inhibits the protein assembly at the ribosome, resulted in an inhibition of the increase of microsomal phospholipid and resulted in an inhibition of the sER proliferation (Orrenius and Ericsson, 1966a). Thus the rER where proteins are synthesized is presumed to be the site of membrane proliferation, and the newly synthesized sER probably arises from the rER. Furthermore, from the cytochemical and biochemical studies on the acyltransferase activity, Higgins and Barnett (1972) have suggested the mechanism of the phenobarbital-induced proliferation of sER as follows: Phospholipid is synthesized in situ at first, and protein is synthesized in the rER and moves to the site of newly synthesized phospholipid, where it is inserted to produce a whole membrane (Higgins and Barnett, 1972).

There are several reports on the fate of the proliferated sER of liver cells induced by chemical agents. The sER proliferation induced in the liver cells of rats by administration of thiohydantoin derivative for 28 days was unobservable by the 5th day after the discontinuation of the drug (Herdson, Garvin and Jennings, 1967). The proliferated sER induced by phenobarbital treatment persisted in the liver cells of rats for up to 15 days after the last of a series of 5 injections (totally 500 mg/kg) (Orrenius and Ericsson, 1966a). Also it persisted in the liver cells of rabbits for 3 weeks after ending the treatment of phenobarbital (totally about 400 mg/kg) (Remmer and Merker, 1965). In the liver cells of dogs, sER proliferation was observed on the 11th day after one injection of 50 mg/kg of phenobarbital or 5 injections of 120 mg/kg of tolbutamide (Remmer and Merker, 1965). Thus an increase in the amount of sER induced by chemical agents persists for various periods after the final treatment with the agents, depending on the kind and the amount of the drug administered. The rates of metabolism and elimination of the drugs may be important factors in the smooth membrane induction, and also in the duration of the induction. Phenobarbital is
eliminated slowly over the course of many days in normal adults, and the rate of disappearance of phenobarbital from plasma has been shown to be 1% per hr in dogs (Maynert, 1971). The half-life of tolbutamide is about 5 hr (Travis and Sayers, 1971), and 90% of tolbutamide is excreted in the urine within 24 hr (Shaw and Beaser, 1971) in normal adults.

In the present investigation, increased amounts of sER were recognized in the cytoplasm of the liver cells even on the 90th day after the last administration of PCB (totally 15 mg/kg of PCB was administered). Bennet (1938) reported that chlorinated biphenyls administered to rats by inhalation in the concentration of 0.57 mg/m³, with exposure 16 hr daily for 134 days, caused liver damage, of which the most striking was hyalinization of the cell cytoplasm, and such cellular alterations essentially persisted 2 months after removal from the exposure. Hyalinization of liver cell cytoplasm observed by light microscopy is comparable to the proliferation of sER by electron microscope (Porter and Brun, 1959; Ortega, 1966).

Kanechlor 400, which was used in the present investigation, contains tetra-, penta-, hexa- and other chlorobiphenyls (Yoshimura and Oshima, 1971). In the mice administered 2 mg of Kanechlor 400 (80 mg/kg), which was dissolved in olive oil and given orally, tetrachlorobiphenyls were scarcely found in their bodies after 3 to 4 weeks, but penta- and hexachlorobiphenyls were still recognized after 10 weeks (Yoshimura and Oshima, 1971). In addition, an enhancement of liver microsomal drug-metabolizing enzyme activity induced by pentachlorobiphenyls was more marked than that induced by tetrachlorobiphenyls, when the same amount was administered to rats (Fujita et al., 1971).

The time course study of the increase in microsomal enzyme activity induced by an administration of 200 mg/kg of tetrachlorobiphenyls to rats revealed that the increase in enzyme activity commenced in 12 hr, reached the maximum a week after the administration, and then was followed by a gradual decline; however such increase was still persistent even 44 days after the administration (Fujita et al., 1971).

The present finding of the sER proliferation 90 days after the cessation of the treatment of PCB may suggest that penta- and hexachlorobiphenyls remain in the guinea pig body for a considerable period and consistently induce proliferation of sER in the liver cells. However, the gradual decrease in the amount of proliferated smooth membranes observed in the present study may suggest that PCB is eliminated gradually from the guinea pig body.

Ultrastructural localization of G–6–Pase in the liver cells of the normal control guinea pigs was almost the same as that reported previously (Ericsson, 1964; Rosen, Kelly and Peters, 1966; Saito and Ogawa, 1967). G–6–Pase activity was also positive in the proliferated sER of the experimental animals observed at any period after the final administration of PCB. G–6–Pase activity was ultracytochemically demonstrated in the proliferated sER in the liver cells of the rats treated with phenobarbital (Orrenius and Ericsson, 1966 b; Saito and Ogawa, 1967), or 3’–MeDAB (Saito, 1968).

Although the specific activity of microsomal G–6–Pase was decreased less pronouncedly but significantly in the rat liver after phenobarbital administration (Orrenius and Ericsson, 1966 b), in contrast it was increased in the rabbits treated with phenobarbital (Remmer and Merker, 1965).
G-6-Pase activity in the rat liver microsome increased slightly on the 2nd day after the administration of tetrachlorobiphenyls, but it decreased thereafter (FUJITA et al., 1971). So cytochemically positive G-6-Pase activity in all the proliferated sER does not necessarily indicate the enhancement of the specific activity of microsomal G-6-Pase in the liver induced by phenobarbital or PCB.

The present finding that G-6-Pase activity was ultracytochemically demonstrated not only in the smooth endoplasmic reticulum in the liver cells of normal controls but also in the proliferated smooth endoplasmic reticulum induced by PCB at any period after the final treatment may suggest that the proliferated smooth membranes are the same in their enzymatic characterization as the normally existing smooth membranes, as far as G-6-Pase is concerned. PCB stored in a guinea pig's body always causes a new synthesis of smooth membranes, into which are incorporated not only drug-metabolizing enzymes but also G-6-Pase, in the liver cells. Therefore, proliferated smooth membranes may have not only the function in drug metabolism, but also the function in glycogen metabolism as normally existing smooth membranes in the liver cell.

References

Proliferation of sER in Liver Cells by PCB


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