

Pericanalicular Rod-Shaped Bodies

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Introduction

In the liver cells intoxicated with carbon tetrachloride (CCl₄), allyl formate and 3'-Methyl-4-Dimethylaminoazobenzene, the unusual structures of rod shaped bodies (RSB) are found in the vicinity of bile canaliculi. These bodies are never seen in the normal hepatocytes. Furthermore, to our knowledge, these bodies have not been reported previously.

In the present report, the ultrastructural details of RSB are described and discussions are made on their morphogenesis and functional significance.

Materials and Methods

A total of 34 male Wistar rats with an average body weight 170 gm, were divided into 6 groups (Table 1). All were fed Oriental basal diet and had free access to water. Group I received 0.5 ml per 100 gm CCl in 50% olive oil solution by intraperitoneal injection. Group II received 0.2 ml per 100 gm CCl₄ in 15% olive oil solution by intraperitoneal injections twice weekly. Group III received 0.4 ml per 100 gm allyl formate in 0.5% olive oil solution by intraperitoneal injection. Animals in group IV were fed 0.06% 3'-Me-DAB in basal diet and those in group V were fed 0.1% alpha-naphthyl isothiocyanate (ANIT) in basal diet. Animals in group VI fed freely basal diet were used as control.

The animals were killed by cervical dislocation on the various periods after treatment as shown in Table 1.

The livers were sectioned and several samples were fixed in 10% formalin for light

Table 1 *Schedule of Treatment for Each Group of Rats*

Group	Number of rats	Duration after intoxication	Treatment
I	4	12 hours	CCl 0.5 ml per 100 gm body weight, by intraperitoneal injection
II	4	2 weeks	CCl 0.2 ml per 100 gm body weight, by intraperitoneal infection twice weekly
II	4	6 weeks	CCl 0.2 ml per 100 gm body weight, by intraperitoneal infection twice weekly
III	4	12 hours	allyl formate 0.4 ml per 100 gm body weight, by intraperitoneal injection
IV	10	140-160 days	feeding with 0.06% 3'-Me-DAB in basal diet
V	4	6 weeks	feeding with 0.1% ANIT in basal diet
VI	4		feeding with basal diet

microscopy. These were embedded in paraffin, sectioned and stained with hematoxylin and eosin and by the periodic acid-Schiff method. A liver tissue for electron microscopy from the right lateral lobe was cut into approximately 1 mm cubes under veronal-buffered 2% osmium tetroxide. After fixation at 4°C for 2 hours, the cubes were dehydrated through graded alcohols and embedded in epon. Sections were cut with a Porter-Blum MT-1 microtome, using glass knives. Ultrathin sections were stained with uranyl acetate and lead hydroxide and examined with a Hitach 11 electron microscope.

Results

RSB in CCl₄ Treated Rat for 2 weeks: A small number of RSB were found in the areas between bile canaliculi and Golgi apparatus which was accompanied by an increased number of coated vesicles. The majority of bile canaliculi were slightly dilated and showed a marked reduction of microvilli in number. An increased number of pericanalicular lysosomes were usually observed (Figs. 1 and 2). RSB were less dense than those of CCl₄ treatment for 6 weeks.

RSB in CCl₄ Treated Rat for 6 weeks: A number of RSB were much more increased and their contents appeared to be denser compared with those of 2 week CCl₄ treatment (Fig. 3). In this stage, most of RSB were found in the immediate vicinity of bile canaliculi which extremely altered with swelling of microvilli and bleb formation. It was distinctly observed that some of RSB are partly limited by a bristled membrane which was identical to a limiting membrane of coated vesicles as shown in the insert of Figure 3.

RSB in CCl₄ Injected Rat for 12 Hours: The dislocation of Golgi apparatus toward nucleus was frequently observed in the pale cells which were induced by acute CCl₄ intoxication¹⁾. Golgi apparatus tended to be vacuolated and scattered. Portions of the Golgi membranes appeared to be a bristled membrane similar to those of the coated vesicles. Around the Golgi zones, an increased number of the coated vesicles and RSB were seen. In these cases, there were seen the transition between RSB and coated vesicles (Fig. 4).

RSB in 3'-Me-DAB Treated Rat: The majority of non-neoplastic hepatocytes had a large number of RSB around the bile canaliculi. The number of RSB in this group were most numerous among those in the animals of 5 groups (Figs. 4 and 5). RSB found in this group, appeared much denser than those in other groups and some of them appeared to be ring or arc shaped (Fig. 6). Bile canaliculi showed the moderate dilatation and the reduction in number of microvilli. Lysosomes and coated vesicles around Golgi apparatus were increased in number.

In the hepatocellular cancer cell with the modified structures of bile canaliculi, none of RSB were observed in the vicinity of canaliculi or any cytoplasmic region²⁾.

RSB in Allyl Formate Injected Rat: RSB were scarcely found in the pericanalicular cytoplasm. The cells revealing little damage in general had RSB, while the cells appeared to be necrotic or severely damaged had no RSB.

Changes in ANIT Treated Rat: The dilatation and the marked reduction of the number of microvilli were seen in the bile canaliculi. Golgi apparatus located nearer to

bile canaliculi than those of normal hepatocytes and pericanalicular lysosomes were increased in number around Golgi zones. However, none of RSB were found in this group (Fig. 8).

General Features of Rod Shaped Bodies

Pericanalicular RSB were found in the hepatocytes from the rats which had received CCl_4 , allyl formate or 3'-Me-DAB. The RSB have never been found in normal and in ANIT treated rat hepatocytes.

RSB were from 45 to 65 $\text{m}\mu$ in width and from 250 to 600 $\text{m}\mu$ in length. The width was fairly constant in 50 μ average, but the length was variable. Some of them reached up to 1.5 μ in length. Occasionally, RSB appeared to be ring or arc shaped. Their contents were quite homogeneous and showed moderate electron density. RSB found in the cells after long term intoxication were, as a rule, denser than those of acute intoxication.

The details of contents occasionally appeared to the parallel-arrayed finely fibrillar structure along the long axis at high magnification (Fig. 10). There was not observed the clear zone between limiting membrane and its content. The limiting membrane was occasionally seen as a bristled membrane. RSB were, in general, found in the vicinity of bile canaliculi, but rarely around the Golgi zones. It is more closely the topographical relation of RSB to the bile canaliculi rather than to the Golgi zones.

In the hepatocytes intoxicated with various hepatotoxins, a marked increase of pericanalicular dense round lysosomes was always observed. In these cells the findings indicating the formation of lysosomes from Golgi apparatus were observed despite of the kind of hepatotoxins used. Some of these lysosomes around the Golgi zones were small and not round (Fig. 9). However, they were readily differentiated from RSB because of the presence of the clear zone between the limiting membrane and unhomogeneous contents.

Discussion

RSB have never been seen in the normal liver cells. The structures corresponding to RSB described in this report have not been reported herein. Accordingly the significance of RSB has been unknown yet. In this paper discussions were made on possible significance of RSB presented.

It seems to be a plausible assumption that RSB are produced as the result of the intracytoplasmic cholestasis since the RSB are usually found in the vicinity of bile canaliculi and the cells which accompanied by the presence of RSB show the alterations of bile canaliculi such as a dilatation of lumen and a decrease in the number of microvilli.

However, the following four facts are contradictory to this speculation. Firstly, RSB are not observed in the cells poisoned with ANIT which has been known to cause a defect in the excretory function of the liver resulting in a cholestasis³⁻⁵. Secondly, the fine structures attributed to the intracytoplasmic congestion of bile are quite different from RSB⁶⁻⁸. Thirdly, the pale cells, which are supposed to be the cells lacking the ability of bile conjugation and excretion, have RSB. Fourthly, the mitotic hepatocytes after partial hepatectomy reveal the appearance of RSB⁹. Judging from these facts,

it is unlikely that there is a relationship between the RSB and intracytoplasmic cholestasis.

Concerning to the functional significance of RSB, the following facts seem to be important. (1) RSB are found in the hepatocytes which always have an increased number of lysosomes. (2) CCl_4 , allyl formate and 3'-Me-DAB which induced the appearance of RSB, are all highly hepatotoxic agents. (3) In the case of 3'-Me-DAB, hepatoma cells which are supposed not being capable to associate with to carcinogenic aminoazo-dye, have no RSB, while most of non-neoplastic hepatocytes which are able to associate with azo-dye, revealed the appearance of RSB. (4) The more chronic intoxication, RSB are, as a rule, increased in number and become dense in their contents. (5) RSB are occasionally located around Golgi zones, but not around bile canaliculi. (6) In the case of the acute intoxication, the cells with little structural damage have RSB, but the cells with severe structural damage have no RSB.

These facts suggest a possibility that RSB may be a structure manifesting for detoxication function of hepatocytes to hepatotoxins.

In this connection, it is thought to be important that some of RSB are occasionally limited partly by a bristled membrane and there are seen the transition between RSB and coated vesicles, though the precise significance of the coated vesicles have also been unknown.

Some investigators reported recently that some of coated vesicles¹³⁻¹⁷) as well as lysosomes^{11,12}) are formed from Golgi apparatus. Some of the coated vesicles show acid phosphatase activities^{13,16,17}). Therefore, some of the coated vesicles are considered to be one of unusual form of primary lysosome. Similar explanation for RSB is considered and no evidence is obtained against this concept for RSB in this study.

It may be possible that RSB contain only one or two kinds of enzymes in striking contrast to the ordinary lysosomes which contain various kinds of enzymes¹⁸). Judging from the constancy of width and the variable length of RSB and from their inner fibrillar structure along their long axis, the materials within RSB may accumulate in the Golgi associated smooth endoplasmic reticulum and may be a simple protein which may develop to one direction. These possible circumstances are noted in the azurophil granules and Auer body in granulocytes¹⁹).

Conclusions

Rod shaped bodies, peculiar structures named by the authors, are found in the hepatocytes intoxicated with CCl_4 , allyl formate and 3'-Me-DAB. The width is fairly constant measured in $50 \text{ m}\mu$. They are mainly located in the vicinity of bile canaliculi. The probable significance of RSB is discussed. It is our assumption to the morphogenesis of RSB that they are produced due to the accumulation of simple lysosomal enzyme in the Golgi associated smooth endoplasmic reticulum and are a kind of the abnormal form of lysosomes.

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Explanation of Figures

- Fig. 1** From the rat 2 weeks after CCl₄ poisoning. Bile canaliculi (BC) are moderately dilated and the number of microvilli is reduced in number. A small number of rod shaped bodies are found near bile canaliculi (arrows). $\times 18,000$.
- Fig. 2** From the rat 2 weeks after CCl₄ poisoning. Rod shape bodies are located near the bile canaliculi (arrows). The number of lysosomes is markedly increased. $\times 18,000$.
- Fig. 3** From the rat 6 weeks after CCl₄ poisoning. Numerous rod shaped bodies are found surrounding the bile canaliculus. The change of bile canaliculus with bleb formation of microvilli are severely. Lysosomes and altered mitochondria are also observed. $\times 32,000$. Insert: Some of rod shaped bodies are limited with a bristled membrane. $\times 46,000$.
- Fig. 4** Pale cell from the rat 12 hours after CCl₄ poisoning. Golgi apparatus, tends to be vacuolated and scattered, located near the nucleus. Coated vesicles and rod shaped bodies are increased around Golgi apparatus. $\times 28,500$.
- Fig. 5** Non-neoplastic hepatocytes from the rat 140 days fed with 0.06% 3'-Me-DAB. Remarkable increases of pericanalicular rod shaped bodies are seen. Bile canaliculus is moderately dilated and the reduction in number of microvilli is also noted. $\times 16,000$.
- Fig. 6** From the same material in Fig. 5. Some of rod shaped bodies are arc or ring shaped. The moderate changes of bile canaliculus are also observed. $\times 16,000$.
- Fig. 7** From the rat 12 hours after allyl formate poisoning. Short rod shaped bodies are seen around the dilated bile canaliculus. Arrows indicate the exocytotic coated vesicles. $\times 28,800$.
- Fig. 8** From the rat 6 weeks after ANIT intoxication. An increase in number of bile canaliculi and dilatation of canaliculi are seen. However, rod shaped bodies are not observed. $\times 11,000$.
- Fig. 9** From the rat 12 hours after allyl formate poisoning. Arc shaped lysosomes (arrows) are seen in the vicinity of Golgi zones. They reveal the clear zones between the limiting membrane and the contents. $\times 22,000$.
- Fig. 10** From the rat 160 days fed with 0.06% 3'-Me-DAB. Ring or rod shaped bodies are seen around Golgi zones. All of bodies are intensively dense. Some of them (arrows) reveal finely fibrillar inner structures. $\times 30,000$.









