

## The toxic effects of bis (tributyltin) oxide on the rat thoracic aorta

M. Yoshizuka<sup>1</sup>, K. Hara<sup>1</sup>, Y. Doi<sup>1</sup>, N. Mori<sup>1</sup>, M. Yokoyama<sup>2</sup>, E. Ono<sup>1</sup> and S. Fujimoto<sup>1</sup>

<sup>1</sup>Department of Anatomy and <sup>2</sup>Laboratory of Electron Microscopy, University of Occupational and Environmental Health, School of Medicine, Kitakyushu, Japan

**Summary.** The toxic effects of bis (tributyltin) oxide (TBTO) on the ultrastructure and permeability of rat thoracic aorta were studied electron microscopically and the accumulation sites of tin were determined with an X-ray microanalyzer.

Male Wistar rats received 0.05ml/kg of TBTO as an emulsion in 1 ml of distilled water through a stomach tube. After time intervals of 2, 4, 6, 8, 10, 12 h after intubation, thoracic aortae were isolated and prepared for electron microscopy.

Marked swelling of mitochondria in the aortic endothelial cells appeared at 4 h after TBTO treatment. By x-ray microanalysis, tin L- $\alpha$  peaks (3.44 keV) were obtained from these swollen mitochondria. Subendothelial edema progressed between 6 and 8 h after TBTO treatment. By tracer experiment, it was seen that large amounts of peroxidase reaction products filled the expanded subendothelial space. At 12 h after TBTO treatment, degenerative changes of the endothelial cells were prominent. These results indicated that orally administered TBTO accumulated in the mitochondria of the endothelial cells of thoracic aorta. The direct toxic effects of TBTO on mitochondria might induce severe damage to the endothelial cells and cause disturbance of the permeability barrier function of the endothelial layer and subendothelial edema.

**Key words:** Aorta, Ultrastructure, Permeability, X-ray Microanalysis, Bis (tributyltin) oxide

### Introduction

In Japan, tributyltin compounds have been widely used in the fishing industry as antifoulants on ships, fish nets, and cages. The recent increase in scoliosis in the cultured yellowtail (*Seriola quinqueradiata*) seems be proportionate to the growing use of these compounds in the fishing industry.

The biological effects of tributyltin compounds were summarized recently (Boyer, 1989; WHO, 1990; Henschler, 1991). However, with regard to the toxic effects of these compounds on the blood circulation systems, no ultrastructural studies have been made.

Therefore, we investigated in detail morphological alterations of rat thoracic aorta after oral administration of bis (tributyltin) oxide (TBTO) using an electron microscope. The accumulation sites of TBTO in the thoracic aorta were examined with an X-ray microanalyzer. Furthermore, the effects of TBTO on the permeability barrier function of the aorta were studied using horseradish peroxidase (HRP).

### Materials and methods

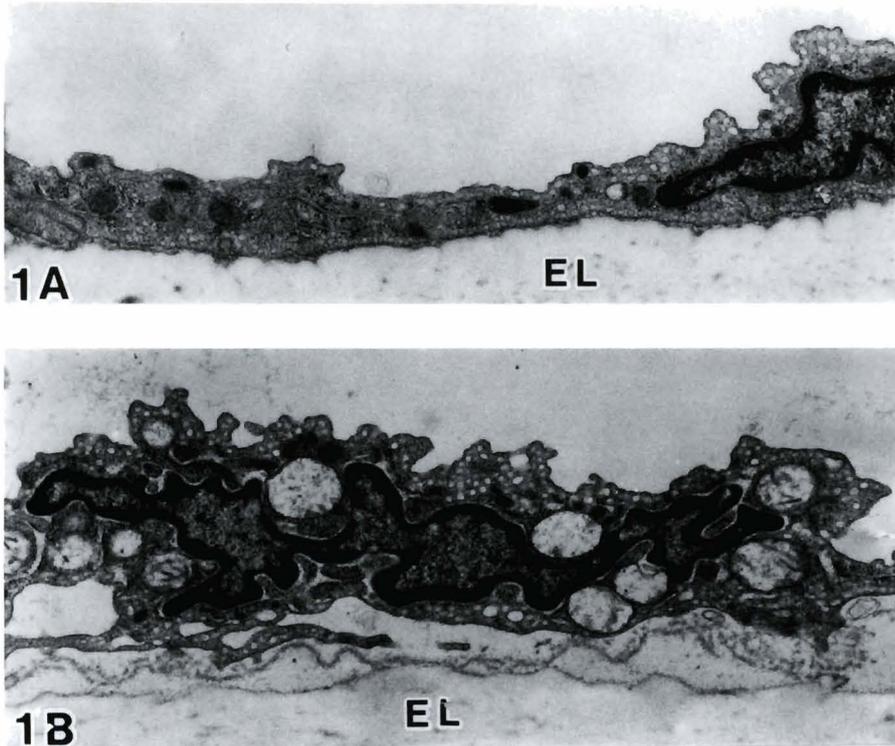
#### Electron microscopy:

Adult male Wistar UOEH rats were housed in individual stainless-steel cages and maintained in a 12 h light dark cycle. Exposure to light was from 06:00 to 18:00 h. The animals completed a 24-h fast prior to dosing.

Twenty-four rats received 0.05 ml/kg body weight of bis (tributyltin) oxide (TBTO; Aldrich Chemical Co. Inc. WI, USA, purity; 96%) orally, as an emulsion in 1 ml of distilled water, through a stainless-steel stomach

Offprint requests to: Dr. Mitsuaki Yoshizuka, M.D., Department of Anatomy, University of Occupational and Environmental Health, School of Medicine, Kitakyushu 807, Japan

*Effects of TBTO on rat aorta*



**Fig. 1A.** An electron micrograph showing the endothelial cells of the control rat thoracic aorta. x 15,000. **1B.** Marked swelling of the mitochondria of the endothelial cell appears at 4 h after TBTO treatment. Subendothelial layer is slightly edematous. x 15,000. EL: elastic lamina.

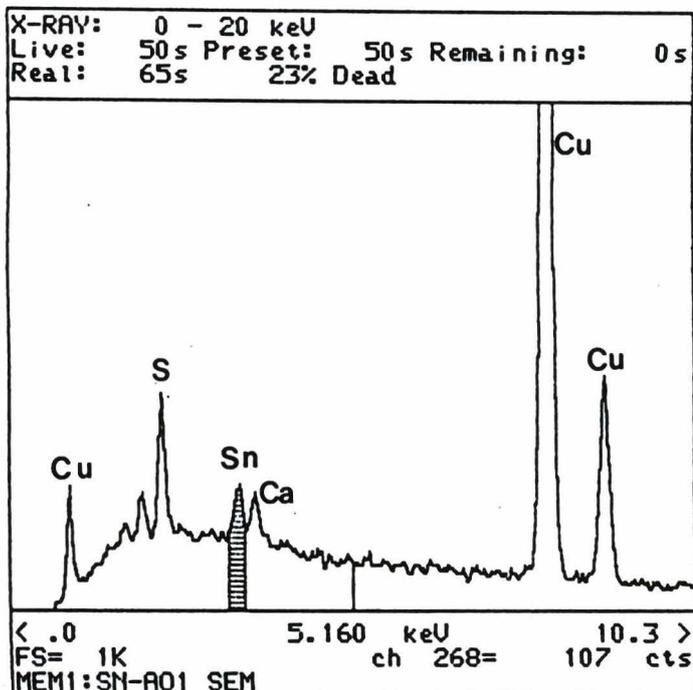
tube. Another twelve rats received 1 ml of distilled water only, and served as controls. After time intervals of 2, 4, 6, 8, 10 and 12 h after treatment, the animals

were deeply anaesthetized by an injection of pentobarbital and the thoracic aortae were isolated. Specimens from all experiments were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4°C for 2 h, postfixed with 2% osmium tetroxide in the same buffer at 4°C for 2 h, dehydrated in a graded series of ethanol, and embedded in epoxy resin.

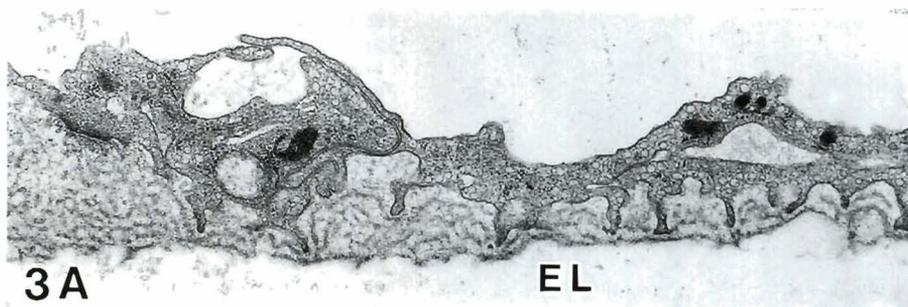
Ultrathin sections were stained with uranyl acetate and lead citrate, and observed using either a JEM 100CX or a JEM 2000EX electron microscope

X-ray Microanalysis:

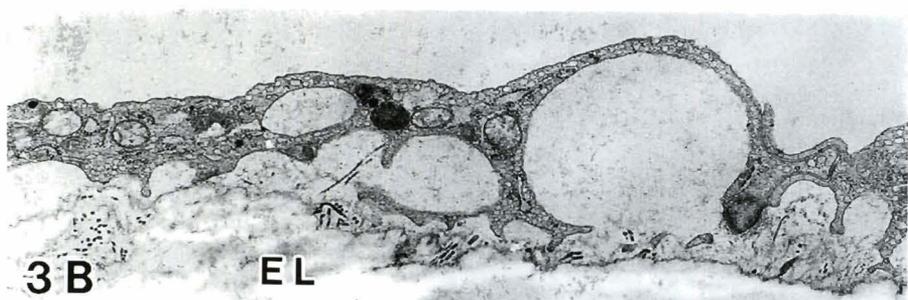
Small pieces cut from the thoracic aorta of all above mentioned animals were immediately frozen in liquid nitrogen without chemical fixation, and freeze-dried in a JEOL JFD 7000 vacuum evaporator. Specimens were analyzed for detection of the accumulation sites of tin in the aorta with a JEM 2000EX electron microscope equipped with a LINK QX 200J energy dispersive X-ray microanalyzer.



**Fig. 2.** An energy dispersive X-ray spectrum from one of the swollen mitochondria in the endothelial cell in Fig. 1B shows an apparent Sn L- $\alpha$  peak at 3.44 keV.



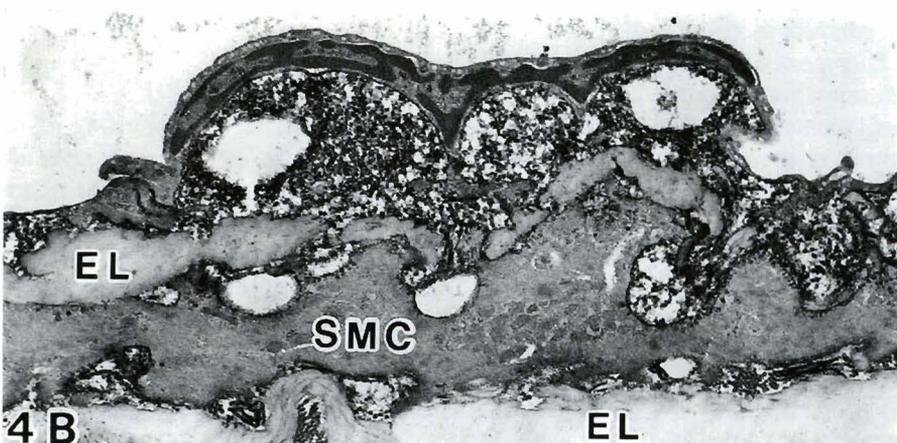
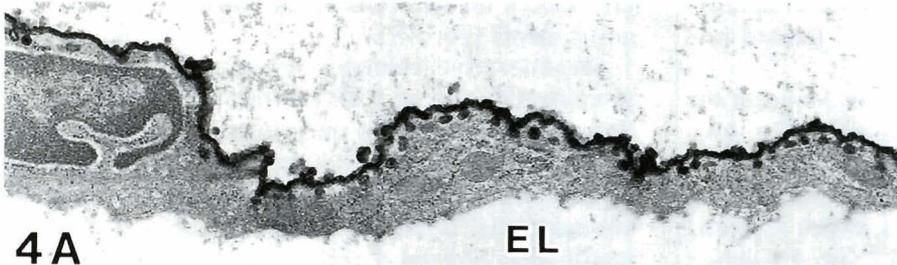
**Fig 3.A.** Inter- and intra-cellular vacuolizations are noted in the endothelial lining at 8 h after TBTO treatment. Subendothelial edema is progressing. x 10,000. **3B.** Attenuation of the endothelial cytoplasm due to the massive influx of plasma into the subendothelial layer is seen at 8 h after TBTO treatment. EL: elastic lamina. x 10,000



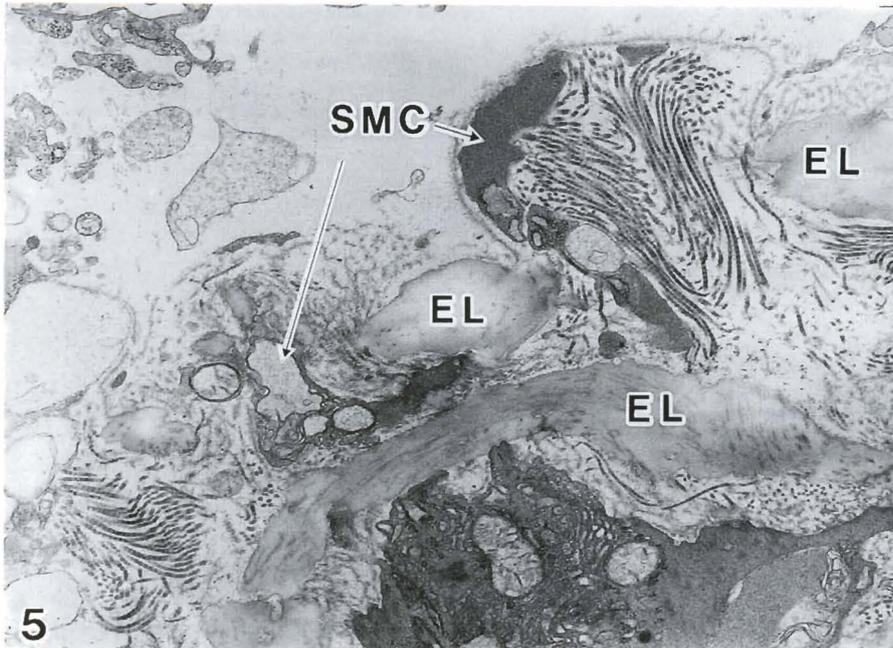
Tracer experiment:

Four rats received 0.05 ml/kg body weight of TBTO as an emulsion in 1 ml of distilled water orally using a stomach tube. At 4 and 8 h after incubation, the animals were deeply anaesthetized by an injection of pentobarbital and received 100 mg/kg body weight of horseradish peroxidase (HRP, Type II, Sigma Chemical Co. St. Louis, MO, USA) dissolved in saline to a final

volume of 0.5 ml by injection into the femoral vein. Two untreated control rats received the same dose of HRP by i.v. injection. Three min. after HRP injection, the aorta was directly perfused with 2.5% glutaraldehyde in 0.1 M cacodylate buffer through the left ventricle of the heart. Then, the thoracic aorta was isolated and further fixed in the same fixative at 4°C for 2 h. The tissues were subsequently incubated for detection of peroxidase at room temperature for 30 min



**Fig. 4A.** Peroxidase reaction products are present within the pinocytotic vesicles of the endothelial cells. However, none is present in the subendothelial layer in the control rat aorta. x 15,000. **4B.** Large amounts of peroxidase reaction products fill the expanded subendothelial space and are found around the smooth muscle cells in the innermost layer of the aortic media at 8 h after TBTO treatment. EL: elastic lamina, SMC: smooth muscle cell. x 10,000.



**Fig. 5.** At 12 h after TBTO treatment, severely damaged endothelial cells are fragmented and desquamated from the endothelial lining. Remnants of degenerated elastic lamina are found in the expanded subendothelial layer. Smooth muscle cells also show degenerative changes to some degree. EL: elastic lamina, SMC: smooth muscle cell. x 12,000

in Graham-Karnovsky medium (0.5 M Tris HCl buffer, pH 7.6, 10 ml, containing 0.01% H<sub>2</sub>O<sub>2</sub> and 10 mg of 3,3'-diaminobenzidine tetrahydrochloride). Specimens were postfixed with 2% osmium tetroxide in 0.1 M cacodylate buffer, and dehydrated, embedded and observed as described above.

## Results

No deaths occurred among the experimental animals associated with TBTO treatment and all treated rats appeared to be in good condition.

No significant structural changes were found in the thoracic aortae at 2 h after TBTO treatment (Fig. 1A). The earliest ultrastructural change attributable to TBTO treatment of the thoracic aorta was the marked swelling of mitochondria in the endothelial cells, which appeared at 4 h after intubation. Their matrices showed a lighter appearance and some of their cristae were destroyed. Subendothelial space became slightly edematous (Fig. 1B). With X-ray microanalysis, tin L- $\alpha$  peaks (3.44 keV) were obtained from these swollen mitochondria of the endothelial cells (Fig. 2). However, such tin peaks were not detected in any other structures in the aortic endothelial cells. Moreover, no tin peaks were obtained from the thoracic aorta of the control rats.

Subendothelial edema progressed between 6 and 8 h after TBTO treatment. Intra- and inter-cellular vacuolizations were noted in the endothelial lining (Fig. 3A). Attenuation of cytoplasm occurred due to the massive influx of plasma into the subendothelial layer.

Degenerative change of internal elastic lamina was also noticed (Fig. 3B).

By tracer experiment, it was seen that peroxidase reaction products were present within the pinocytotic vesicles of the endothelial cells. However, none were present in the subendothelial space in the control rat aorta (Fig. 4A). At 8 h after TBTO treatment, large amounts of peroxidase reaction products filled the expanded subendothelial space and were found around the smooth muscle cells in the innermost layer of the aortic media (Fig. 4B).

Degenerative changes of endothelial cells were prominent at 12 h after TBTO treatment. Some severely damaged endothelial cells were fragmented and desquamated from the endothelial lining. Remnants of degenerated elastic lamina were found in the expanded subendothelial layer. Smooth muscle cells in the aortic media also showed a certain degree of degenerative change (Fig. 5).

## Discussion

In the present experiment, marked swelling of the mitochondria in the endothelial cells appeared as the earliest ultrastructural change of the thoracic aorta attributable to the TBTO treatment. The energy dispersive X-ray microprobe data indicated that administered TBTO accumulated in these swollen mitochondria. Swelling of mitochondria in the kidney (Wakashin, 1975), cornea (Yoshizuka et al., 1991) and liver (Yoshizuka et al., 1992), caused by TBTO

intoxication, has been reported. It is known that organotin compounds inhibit the oxidative phosphorylation of mitochondria in vitro (Aldridge, 1958; Stockdale et al., 1970). The swelling of mitochondria, which was observed in the endothelial cells of TBTO-treated rat aorta, was probably the morphological manifestation of the mitochondrial dysfunction due to the toxic effects of TBTO.

Subendothelial edema occurred in TBTO-treated rat aorta. The findings of the present tracer experiment indicate that the increase in permeability of aortic endothelial cells is induced by TBTO treatment. Since vascular endothelial lining acts as a permeability barrier, a damage to the endothelial cells caused subendothelial edema. It seems that the direct toxic effects of TBTO on mitochondria might induce severe damage to the aortic endothelial cells and cause disturbance in the permeability barrier function of the endothelial layer, followed by edematous swelling of subendothelial space. A massive influx of plasma into the subendothelial layer might cause the expansion of this layer, intra- and inter-cellular vacuolizations in the endothelia and degenerative changes of internal elastic lamina and medial smooth muscle cells.

This is the first report on the fine structural of vascular endothelial cells as a systemic effect of TBTO. Morphological studies on the systemic effects of TBTO in the microcirculation of various

organs are now in progress in our laboratory.

## References

- Aldridge W.N. (1958). The biochemistry of organotin compounds. Trialkyltins and oxidative phosphorylation. *Biochem. J.* 69, 367-379.
- Boyer I.J. (1989). Toxicity of dibutyltin, tributyltin and other organotin compounds to humans and experimental animals. *Toxicology* 55, 253-298.
- Henschler D. (1991). Tri-n-butyltin compounds. In: Occupational toxicants: critical data evaluation for MAK values and classification of carcinogens. Vol. 1, Henschler D. (ed). VCH Verlagsgesellschaft mbH. Weinheim. pp 315-333.
- Stockdale M., Dawson A.P. and Selwyn M.J. (1970). Effects of trialkyltin and triphenyltin compounds on mitochondrial respiration. *Eur. J. Biochem.* 15, 342-351.
- Wakashin K. (1975). Experimental studies on the toxicity of organic tin compounds - morphology of the butyl-tin intoxication Tokyo Ikadaigaku Zasshi 33, 573-596 (in Japanese).
- WHO (1990). Tributyltin compounds. Environmental Health Criteria Vol. 115. World Health Organization, Geneva.
- Yoshizuka M., Haramaki N., Yokoyama M., Hara K., Kawahara A., Umezu Y., Araki H., Mori N. and Fujimoto S. (1991). Corneal edema induced by bis (tributyltin) oxide. *Arch. Toxicol.* 65, 651-655.
- Yoshizuka M., Hara K., Haramaki N., Yokoyama M., Mori N., Doi Y., Kawahara A. and Fujimoto S. (1992). Studies on the hepatotoxicity induced by bis (tributyltin) oxide. *Arch. Toxicol.* 86, 182-187.

Accepted April 1, 1992