usually disappears within 5 min. Prior administration of either atropine (50 nmoles) or hexamethonium (100 nmoles) delayed the appearance of tremor (induced by 50 nmoles of physostigmine) by 4–10 min, but neither agent reversed the effects of physostigmine when given after the esterase inhibitor. We conclude that nicotine's cholinergic action is necessary but not sufficient to explain the prostration syndrome.

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Degeneration of retinal neuroblasts by chinoform-ferric chelate[1]

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Summary. The possible mechanism of neuropathic effect of chinoform was investigated using cultured retinal neuroblasts from chick embryos. Retinal neuroblasts completely degenerated by chinoform-ferric chelate within a day. This change, however, was not observed with free chinoform or ferric ion. *a*-Tocopherol had a potent protective effect on the toxicity of the chelate. From these results, it was concluded that the lipid peroxidation due to ferric ion chelated with chinoform incorporated into the membrane of nerve tissues is the most important step in induction of the neuropathy.

It was revealed by epidemiological research that subacute myelo-optico-neuropathy (SMON) was caused by massive doses of chinoform (5-chloro-7-iodo-8-quinolinol)[3,4]. Chinoform administered is known to be absorbed and incorporated into various organs including nerve tissues[5,6] and green-colored substance, appearing on the tongues and in the urine and feces of SMON patients, was identified as chinoform-ferric chelate[7]. In 1976, Yagi et al.[8] reported the effects of chinoform and chinoform-ferric chelate on an isolated sciatic nerve and found that chinoform-ferric chelate increased the lipid peroxides in the nerve sample, but free chinoform had no effect. They predicted that the lipid peroxidation initiated by chinoform-ferric chelate is the direct cause for the degeneration of the nerve tissues. To confirm this supposition, we intended to examine the toxicity of chinoform-ferric chelate on cultured retinal neuroblasts in comparison with free chinoform. The protection with an antioxidant, *a*-tocopherol, was also examined.

Materials and methods. Neural retinal cells were prepared by the method of Okada et al.[9]. Approximately 1.5×10^7 cells were inoculated into each Falcon plastic culture dish (35 mm in diameter) using 1.5 ml of a culture medium consisted of Eagle's minimum essential medium (MEM, NISSUI, Tokyo) supplemented with 8% fetal bovine serum (Gibco, New York), 0.3% L-glutamine and 0.14% sodium bicarbonate. The same lot of serum was used during the experiments. These culture dishes were incubated at 36.5 °C under an atmosphere of 5% CO_2-95% air. The culture medium was changed every 2 days for 5–6 days until the test. Chinoform and DL-*a*-tocopherol were sonicated in the medium to be emulsion. Chinoform-ferric chelate was prepared by sonicating the suspension of chinoform and $FeCl_3$ (molar ratio of chinoform to ferric ion, 10:3) in the medium. Morphological observation of the cells was made using an inverted phase contrast microscope (Zeiss, Invertoscope D).

To check the cell degeneration, dye exclusion test was carried out. After incubation of the cells with various reagents for a definite period, 0.2 ml of 0.5% erythrosine B in Ca^{2+}- and Mg^{2+}-free Hanks' solution (CMF) was added to a culture dish. 5 min later, the dish was fully washed with CMF 8–10 times. The cells were dissolved in 2.5 ml of 0.5 M KOH, and erythrosine B incorporated into the denatured cells was estimated by measuring the absorbance at 529 nm.

Results. Within 1–2 days after inoculation of cells, aggregates of cells adhered to culture dish. Thereafter, cells in aggregates started to spread. Aggregates were often interconnected with long axonal processes. At about 5 days, a sheet of flatened epithelial cells was formed, upon which small neuroblasts with axonal processes were superimposed (fig. 1, A). Epithelial cells were very thin, well-spread, transparent and poorly refractile under phase optics. On the other hand, neuroblasts were highly refractile due to their dense cytoplasm. Therefore, it was easy to distinguish neuroblasts from epithelial cells[9]. When 50 µM of chinoform-ferric chelate was added to neural retinal cells, degenerated neuroblasts were not observed until 3 h of incubation, and neuroblasts stained with erythrosine B were not detected. Some neuroblasts degenerated at 5 h (fig. 1, B), and at that time a slight increase of erythrosine B incorporation was observed (fig. 2). Then the incorporation of the dye into degenerated neuroblasts increased rapidly and the maximum incorporation was observed at 10 h after the addition of 50 µM of the chelate (fig. 2). These degenerated neuroblasts began to detach from a sheet of epithelial cells during further cultivation of these cells under the same condition, and cell debris floating in culture medium could be seen. Since the detached cells were removed from the dish by washing, incorporation of erythrosine B was decreased gradually from 10 to 24 h (fig. 2). Some degenerated cells still attached to epithelial cell sheet were

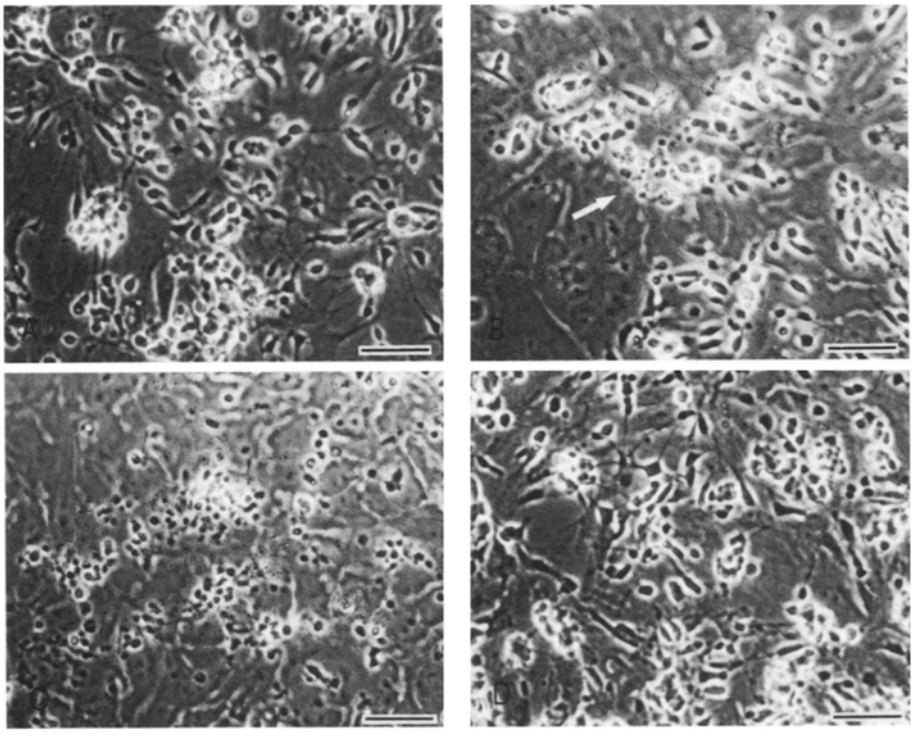

Figure 1. Effect of chinoform-ferric chelate on retinal neuroblasts. *A* Control cells, no addition. *B* Cells at 5 h of incubation in the presence of 50 μM of chinoform-ferric chelate. Some degenerated and shrunk neuroblasts could be seen (arrow shows). *C* Cells at 24 h of incubation under the same condition of fig. 1, B. Almost all neuroblasts in this picture degenerated and shrank. Epithelial cells, however, did not degenerate. *D* 50 μM of chinoform-ferric chelate and 5 μM of α-tocopherol were added to the culture dishes and incubated for 1 day. Phase contrast optics (scale: 50 μm).

observed at 24 h after the addition of the chelate (fig. 1, C). Further cultivation of these cells under this condition for 2–3 days resulted in the detachment of almost all degenerated neuroblasts from epithelial cell sheet. In this experiment, degeneration caused by chinoform-ferric chelate was observed with neuroblasts, but not with epithelial cells (see fig. 1, C). Since epithelial cells could not be stained by erythrosine B after cultivation of the cells in the presence of 50 μM of the chelate for 1 week, it can be concluded that these epithelial cells were very resistant to the toxicity of the chelate in contrast with neuroblasts.

On the other hand, no significant change was observed when the cells were cultivated in the presence of either 50 μM of free chinoform or 15 μM of ferric ion. This observation was confirmed by dye exclusion test. As shown in figure 2, absorbance of erythrosine B of the cells incubated in the presence of either 50 μM of free chinoform or 15 μM ferric ion was similar to that of control cells during 24 h of cultivation.

When neural retinal cells were cultured in the presence of 50 μM of the chelate together with 100 μM α-tocopherol for 1 day, erythrosine B staining was not observed (see fig. 2), and there was no significant morphological difference between these cells and control cells. After further cultivation under this condition for 3 days, cell degeneration was scarcely observed. Highly potent protective effect of α-tocopherol on the toxicity of the chelate was observed with even less amount of α-tocopherol: in the presence of 5 μM of α-tocopherol and 50 μM of the chelate, no significant morphological change of neuroblasts was found (fig. 1, D), and the cells were not stained with erythrosine B. When the cells were cultured in the presence of 100 μM of α-tocopherol for 1 day, and then these cells were fully washed with CMF 8–10 times and cultivated in a fresh medium containing 5–50 μM of the chelate for 1–3 days, degeneration of neuroblasts could be hardly recognized. Another antioxidant, butyrated hydroxy toluene (10 μM), had also protective effect, but was less effective than α-tocopherol.

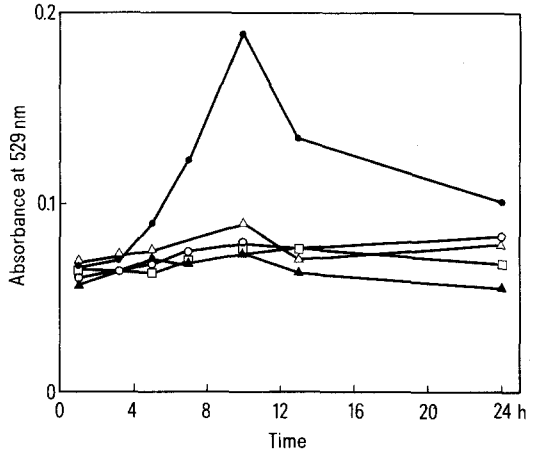

Figure 2. Time course of incorporation of erythrosine B into degenerated retinal neuroblasts. See materials and methods for details. ○, Control cells, no addition; ●, 50 μM chinoform-ferric chelate; △, 50 μM free chinoform; ▲, 15 μM ferric chloride; □, 50 μM chinoform-ferric chelate plus 100 μM α-tocopherol. Each point represents the mean of 4 culture plates. SE of each point is less than 15% of each value. Even if there are no degenerated cells, some absorbance at 529 nm is observed as can be seen with control cells. This means nonspecific attachment of the dye to culture dish and cells.

Discussion. In the present study, it was found that degeneration of cultivated neuroblasts was caused by chinoform-ferric chelate, but not by either free chinoform or ferric ion, and that the degeneration could be protected by the coexistence of α-tocopherol. Taking into account our previous result[8] that chinoform-ferric chelate provoked the increase of lipid peroxide level of the isolated sciatic nerve, the observed protective effect of α-tocopherol would be ascribed to its antioxidant action.

It is well known that the proteins and membranes are damaged by lipid peroxidation[10]. We observed that upon exposure of rabbit to high concentration of oxygen, lipid peroxides in the retina were increased with the decrease in electroretinogram and morphological change of the retina[11]. When chick embryo was exposed to high concentration of oxygen, lipid peroxides in the blood were increased and then those in the retina were increased accompanying with its morphological change[12]. These results indicate that the increase in lipid peroxides induces the degeneration of retinal cells. Considering these results, it was supposed that the mechanism of the presently observed deleterious effect of chinoform-ferric chelate on retinal neuroblasts would be due to the initiation of lipid peroxidation by iron transferred into the lipid layer of membranes of the cells by hydrophobic nature of chinoform. However, the fine mechanism should await further investigation, which is under progress in our laboratory.

There was remarkable difference in susceptibility to the toxicity of chinoform-ferric chelate between neuroblasts and epithelial cells. Our preliminary experiment showed that, when 50 μM of chinoform-ferric chelate was added to the culture medium of cerebrum, skeletal muscle and skin fibroblast cells, nerve cells and skeletal muscle cells degenerated but not other cells. Moreover, when linoleic acid hydroperoxide was directly added to the culture medium of neural retinal cells, neuroblasts were more sensitive to the hydroperoxide than epithelial cells. From these observations, it is suggested that the cells with excitable membrane such as nerve and muscle cells were more sensitive to the lipid peroxides than other non-excitable cells. Recently, Prasad et al.[13] reported that neuroblastoma cells were more sensitive to sodium ascorbate than glioma cells by the criterion of growth inhibition due to cell death and reduction of cell division. They suggested that sodium ascorbate led to the production of H_2O_2 which in turn induced lipid peroxidation. Accordingly, the difference in the susceptibility would be related to the mechanism of formation and elimination of lipid peroxides in these cells.

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Smooth muscle cells in 'venous patches' grafted into the rat common carotid artery. A structural study[1]

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Summary. In the 2nd week after surgery, well differentiated smooth muscle cells (SMC) were evident in the walls of venous patches in rat common carotid artery. Gap junctions were the only type of intercellular junction observed between SMC in the present study.

In the rat saphenous vein[4] a basal lamina separates the endothelium from the smooth muscle cells, which are distributed in 2 concentric layers perpendicular to the main axis of the vessel. Smooth muscle cells (SMC) are interconnected by gap junctions. Elastic fibers, parallel to the blood flow, are scattered between SMC. The most external part of the venous wall is formed by 3 fibroblast layers concentrically oriented towards the vascular lumen. Collagen fibers are present, running along the vein axis between fibroblasts. Inmediately following grafting of an autologous strip of saphenous vein (venous patch) into the common carotid artery in the rats, venous tissues undergo great alterations in response to ischemia and surgical trauma: the endothelium desappears by desquemation and the SMC and fibroblasts degenerate. After surgery, the wall of the venous patch is formed by cellular remains at different degenerating stages and a red thrombus.

Muscular hyperplasia is a constant phenomenon in the walls of small vessels submitted to different microsurgical techniques[3]. The origin of the smooth muscle cells (SMC) which are present in the fibromuscular hyperplasia of small vessels operated on with microsurgical techniques has not yet been explained, and the mechanism of muscular proliferation is also controversial. In grafted venous patches in the rat common carotid artery, a fibromuscular hyperplasia is evident at the end of the 1st week after surgery[4]. The purpose of the present paper was the ultrastructural study of SMC in venous patches grafted into the right common carotid artery of the rat during the period when fibromuscular hyperplasia is detectable with a light microscope.

Material and methods. In this study, 35 Wistar rats, averaging 200 g were used. With the help of an operating microscope, a 10–12 mm long segment of the saphenous vein was extirpated and longitudinally incised on a rectangular strip that was immediately placed in a saline solution. The right common carotid artery was then dissected and clamped as caudally and cranially as possible with Scoville clips, and a 5–8 mm longitudinal arteriotomy was performed. The autologous venous patch was grafted onto the edges of the carotid incision with interrupted sutures of 10-0 Nylon monofilament. 10–20 days after surgery, the animals were anaesthetized with i.p. Nembutal and fixed by