The results are summarized in the Table. Among the anticonvulsants 2-propylpenten-2-oic acid and 5-propyloctanoic acid were found to be relatively weak inhibitors of GABA uptake by synaptosomes. These compounds had no effect on uptake of GABA by brain slices. None of the other anticonvulsant fatty acids, including nDPA, influenced uptake by either synaptosomes or brain slices; 2-Butyl-3-propylhexanoic acid was the only compound which significantly inhibited uptake of GABA by both preparations. Kinetics of the inhibition of uptake by synaptosomes was studied at 4 different GABA concentrations (3.12 μM, 4.17 μM, 6.12 μM and 12.50 μM) and 2 different inhibitor concentrations (250 μM and 500 μM). The inhibition was found to be of a linear, non-competitive type with $K_i = 620$ μM. It is possible that a slower diffusion rate of 2-pentyl-3-propylhexanoic acid is responsible for a smaller amount of inhibition observed in brain slices as compared to that by synaptosomes. This can also explain lack of effect of 5-propyloctanoic acid and 2-propylpenten-2-oic acid on uptake by brain slices.

In summary, the results reported in this communication show no correlation between anticonvulsant properties of the compounds and their potency as inhibitors of high affinity uptake of GABA.

Formation of Lipoperoxide in Isolated Sciatic Nerve by Chinoform-Ferric Chelate

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Summary. Effects of chinoform and chinoform-ferric chelate on formation of lipoperoxide in isolated sciatic nerve were investigated. Free chinoform did not increase the lipoperoxide level, while chinoform-ferric chelate significantly increased it. Assuming that the lipoperoxide formed denatures the associated protein in the nerve, the effect of chinoform-ferric chelate could explain, at least partly, the demyelination of nerve tissues caused by massive doses of chinoform.

Epidemiological investigation in Japan revealed that massive doses of chinoform (5-chloro-7-iodo-8-quinolinol) cause a neuropathy called subacute myelo-optico neuropathy (SMON)[2,3]. Although administered chinoform is known to be absorbed in the animal body and found in nerve tissues[4,5], the mechanism of provocation of the neuropathy has not yet been fully elucidated.

Assuming that chinoform provokes the neuropathy through its direct effect on nerve tissue in situ, in vitro experiments to study the effect of chinoform on nerve tissue were considered worthwhile. It was found that the green-colored substance which appears on the tongues and in the feces of SMON patients (recognized as a characteristic symtom of this disease), is due to chinoform-ferric chelate[6]. Thus the investigation of chinoform-ferric chelate is also needed. Assuming that the morphological changes found in the nerves as a result of massive doses of chinoform are due to the denaturation of protein moiety of nerve tissues, we investigated whether chinoform or chinoform-ferric chelate has an action in denaturing the protein in the nerves. On this assumption, we have a working hypothesis that the effect of chinoform is directed primarily to lipid moiety of nerve tissue to increase its peroxide. If lipoperoxide is formed in the tissue, it would cause denaturation of associated protein, resulting in the degeneration of nerve tissue. To examine this working hypothesis, the present work deals with the measurement of lipoperoxide in isolated sciatic nerve dipped into sonicated emulsion of free chinoform or chinoform-ferric chelate in 0.9% NaCl aqueous solution.

Materials and methods. Crystalline chinoform was kindly donated by Prof. Z. TAMURA of Tokyo University. Chinoform-ferric chelate was prepared by mixing $FeCl_3$ with chinoform in dichloromethane and purified according to Tamura et al.[6].

The sciatic nerves of male adult rabbit were extracted very carefully and cut into pieces of 2.5 cm length and about 100 mg wet weight. The nerve was put into test medium except the top and the tail portions, thus forming a U-shape.

Since the level of chinoform accumulated in the sciatic nerve of mice 2 days after the injection of 100 mg/kg of chinoform was reported to be 1.6 μg/g[5], much higher concentration of chinoform was used. As test medium, 2.5 mM chinoform or the same concentration of chinoform-ferric chelate was suspended in 0.9% NaCl aqueous solution and sonicated in an ice-bath to be emulsion. The nerves were incubated at 5 °C for 4 days in the dark. Nerve pieces of the same length and weight in 0.9% NaCl aqueous solution were adopted as control.

Effects of chinoform and chinoform-ferric chelate on formation of lipoperoxide in isolated sciatic nerve

Lipoperoxide value		
control	+ chinoform	+ chinoform-ferric chelate
1.85 ± 0.45	2.06 ± 0.12	3.28 ± 0.98

Lipoperoxide value was represented by nmoles of malonaldehyde determined by TBA method (see text). Numbers of experiments are 4. Statistically significant increase was observed both between control and chinoform-ferric chelate ($p < 0.10$) and between chinoform and chinoform-ferric chelate ($p < 0.10$).

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As another control, nerve pieces were incubated in the aqueous solution of 0.8 mM of $FeCl_3$, where concentration of ferric ions is identical to the total iron concentration of the chelate, 2.5 mM (molar concentration of chinoform to ferric ion, 3:1).

After incubation, the nerves were washed with 0.9% NaCl aqueous solution, and lipoperoxide contents were determined by thiobarbituric acid (TBA) method[7] modified by Nishigaki et al.[8]. The nerve piece was placed in 2.0 ml of 0.9% NaCl, and 1.0 ml of TBA reagent (the mixture of equal volumes of 0.67% TBA aqueous solution and glacial acetic acid) and 2.0 ml of H_2O were added. This was heated at 95 °C for 1 h in an oil bath. After cooling with tap water, the reaction mixture was shaken with 5 ml of chloroform and centrifuged at 3,000 g for 10 min. The supernatant was centrifuged further at 10,000 g for 10 min and the clear supernatant was subjected to absorbance measurement at 532 nm. The amounts of lipoperoxide were expressed in terms of nmoles of malonaldehyde, which were calculated from the value for tetraethoxypropane treated in the same way as above[9].

Results and discussion. The results are summarized in the Table. As compared with control, chinoform does not significantly increase the lipoperoxide level in the nerves, while chinoform-ferric chelate increases it ($p < 0.10$). Since it is well known that ferric ion has the action to provoke lipoperoxide, the effect of ferric ions, the concentration of which is identical to the total iron concentration of the chelate, was examined. In this case, the increase in the lipoperoxide level was 8 times higher than that in the case of the chelate. Accordingly, we naturally wondered whether the action of the chelate could be ascribed to chinofrom-ferric chelate itself or to ferric ions partly liberated from the chelate in the sonicated medium. To check this point, the following experiments were made. First, the liberation of ferric ions from the chelate in the sonicated medium was examined. From the sonicated medium, the chelate and free chinoform were extracted with dichloromethane and the remaining ferric ions were determined by α,α'-dipyridyl. The result showed that the amount of dissociated ferric ions was less than 1/7,000 of the total iron. Second, the effect of 0.8 μM of $FeCl_3$ on isolated sciatic nerve was examined under the same conditions. The result showed no significant increase in lipoperoxide formation. This concentration of ferric ions corresponds to that of 1/1,000 of iron contained in 2.5 mM chinoform-ferric chelate. Accordingly, the action observed with chinoform-ferric chelate could not be ascribed to ferric ions, but to the chelate itself. Therefore, the direct interaction between chinoform-ferric chelate and sciatic nerve is considered to be the initial event in the provocation of the lipoperoxide formation mentioned above, but still it is not clear in the nerve tissue whether chinoform-ferric chelate itself actually provoked the lipoperoxide formation or ferric ions liberated from the chelate in situ did. In any way, it can be predicted that chinoform administered to animal body forms its ferric chelate (or other metal chelate) and is incorporated into nerve tissues and that the chelate provokes the formation of lipoperoxide, resulting in the denaturation of the protein in situ. This seems to be one of the mechanisms for demyelination of the nerves caused by massive doses of chinoform. To ascertain this prediction, in vivo investigations are in progress in our laboratory.

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The Electrophysiological Effects of Ionophore X-537A on Cardiac Purkinje Fibres[1]

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Summary. Using classical microelectrode techniques in canine cardiac Purkinje fibres, calcium ionophore X-537A was shown to shorten the action potential, hyperpolarizing the membrane and lowering the plateau, suggesting that intracellular calcium controls membrane permeability to potassium in this preparation.

Certain carboxylic ionophores transport calcium and other cations across lipid barriers such as cell membranes[3]. Because the ionophores increase ionized intracellular calcium concentration (Ca_i)[4] they may be useful as inotropic agents[5] and as 'tools' with which to study the effects of Ca_i on the electrical and/or mechanical properties of excitable cells[6]. The present study was undertaken to determine the effects of ionophore X-537A (Hoffmann-La Roche, Inc.) on the action potential of canine cardiac Purkinje fibres.

Methods. Strands of Purkinje fibres (0.5–0.8 mm in diameter and 5–10 mm in length) are removed from the ventricles of mongrel dogs, 15–18 kg, anesthetized with 30 mg/kg Nembutal, and mounted in a Plexiglas tissue bath with a volume of 1.0 ml described in detail elsewhere[7]. The tissue is superfused at a rate of 15–20 ml/min with modified Tyrode's solution. The solution is equilibrated with 95% O_2–5% CO_2 resulting in a pH of 7.2–7.4. The temperature of the fluid is maintained at 37 $\pm$ 0.2 °C. In the experiments in which lanthanum chloride is added, the buffer system ($NaHCO_3$, NaH_2PO_4) is replaced by HEPES-NaOH buffer. A stock solution of the ionophore is made using dimethyl sulfoxide solvent at a concentration of 5×10^{-2} M. The amount of dimethyl sulfoxide in the final solution has no electrophysiologic effect on cardiac

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