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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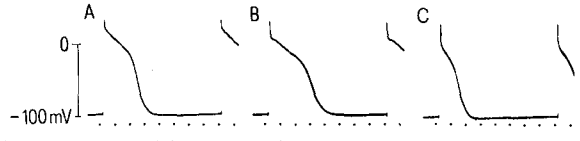
Purkinje fibres (personal observations). Electrical recording is achieved by standard microelectrode techniques[8].

Results and discussion. In a series of 26 experiments the ionophore shortened the action potential duration whether measured at 50%, 75%, 90% or 100% of repolarization. The ionophore decreased the level of the plateau whether measured 50, 100, 150 or 200 msec after onset of the action potential. In most experiments the ionophore hyperpolarized the membrane and the preparation became less easily excitable by an external stimulus. Exposure of the preparation to lanthanum chloride (50 μM), verapamil (1 mg/l), propranolol (10^{-6} M), or practolol (10^{-4} M) did not inhibit these effects of X-537A on the action potential.

Effect of X-537A on a canine Purkinje fibre preparation

	Overshoot (mV)	Plateau height[a] (mV)	Action potential duration[b] (msec)	Maximum diastolic potential (mV)
Control	+ 34	+ 2	322	— 94
Practolol	+ 27	— 5	424	— 93
X-537A	+ 23	— 31	209	— 95

[a] Level of the plateau with respect to 0 mV measured 100 msec after onset of the upstroke of the action potential. [b] Measured from the onset of the upstroke of the action potential to 100% repolarization.



The effect of X-537A on a Purkinje fibre.
A) Control, external stimulation at 1.2 Hz. B) After exposure to practolol 10^{-4} M for 137 min. C) After exposure to X-537A 10^{-5} M for 50 min. The external potassium concentration was 4 mM per liter. 0 mV and —100 mV are indicated by the vertical calibration. The horizontal dots are 100 msec apart.

The Figure shows the typical effect of X-537A (10^{-5} M) on a canine Purkinje fibre preparation that had been superfused for 137 min with practolol (10^{-4} M). Panel A) is the control action potential. Panel B) is the action potential after exposure to practolol (10^{-4} M) for 137 min. Panel C) shows the action potential after exposure to X-537A (10^{-5} M) for 50 min. The Table summarizes the result of this experiment.

The change in the overshoot (Figure and Table) may be due to an ionophore-induced increase in intracellular sodium concentration (Na_i) or the decrease in overshoot may be due to practolol exposure. The decrease in plateau potential is due to the increase in outward potassium current and to the decrease in inward driving force on calcium. These effects are due to the ionophore-induced increase in Ca_i. The dramatic shortening of the action potential duration results from the Ca_i-induced increase in potassium permeability. The slight ionophore-induced increase in maximum diastolic potential is not significant in this experiment but in other experiments performed with potassium concentrations of 2.7 mM or 2.0 mM, ionophore-induced increase in Ca_i caused significant hyperpolarization of the membrane.

In spontaneous preparations, X-537A suppressed spontaneous activity. The absence of calcium prevented the ionophore effect. Purkinje fibres of a dog pretreated with reserpine (total dose 1.2 mg/kg divided over 4 days) were exposed to practolol (10^{-4} M) and X-537A (10^{-5} M). The effect of X-537A on this preparation was identical to the effect on the preparation shown in the Figure.

These results tend to confirm the suggestion that Ca_i modulates potassium permeability in cardiac tissues[6,9] as it has been shown to do in other excitable cells[10,11].

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Role of 5-Hydroxytryptamine in Prostaglandin E_1-Induced Potentiation of Hexobarbitone Hypnosis in Albino Rats

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Summary. PGE_1 potentiated, while diclofenac, a prostaglandin synthesis inhibitor, antagonized hexobarbitone hypnosis in rats. PGE_1-induced potentiation of hexobarbitone sleep was inhibited by a 5HT synthesis inhibitor and by a 5HT receptor blocker, suggesting that this potentiation is 5HT mediated.

Prostaglandins are present in brain and other organs of mammals and are extremely active in a number of biological systems[2-4]. Prostaglandins of the E series have been shown to cause profound sedation, stupor and catatonia, when administered intraventricularly to cats or i.v. to chicks[5-7]. In a recent study[8], intraperitoneal administration of prostaglandin E_1 (PGE_1) was shown to produce marked sedation in rats, to the extent of producing a behavioural state superficially resembling normal sleep with, however, an EEG pattern seen in normal waking state. This PGE_1 induced sedation was accompa-

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