The ultrastructural and cytochemical analyses lead us to suppose that this structure would correspond to the NOR undergoing a collapse within the lacunar areas of the nucleolus by the action of the drug, as has been reported with other drugs which interact with nucleolar activity[19,20]. After the nucleolar degranulation and later disappearance of the nucleolus as an organized structure, the NOR would preserve its conformation, in the same way as the perinucleolar masses of condensed chromatin do, not occupying the space previously filled by the nucleolus. This would indicate that continuous EB treatment may produce a rigidity in the nucleolar as well as in the extranucleolar chromatin, as other authors[21-23] and we[9] have previously reported. The ribonucleoproteic structures with a lower contrast, which appear filling the spaces in among the groups of nucleolar chromatin fibres, would correspond to remnants of altered nucleolar material related to the fibrillar zone[12] which would be trapped by the NOR's mesh. The collapse of the intranucleolar chromatin areas is a morphological effect of the inhibition of DNA-directed RNA synthesis different to the appearance of fibrillar bodies.

These bodies, which appear in Allium cepa[24,25] and also in animal cells[26] after RNA synthesis inhibitor treatments, have also been described as a constant component of the nucleolus[27], and seem to correspond to dispersed chromatin[27,28]. Under EB these bodies do not show a collapsed aspect, as does the intranucleolar chromatin, perhaps because of their high protein content[24,25,27] which would make them in accessible to the EB, or perhaps because, as we postulated previously[24,25], they are inactive organelles not involves in active cellular metabolism.

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Inhibition by verapamil of ionophore-mediated calcium translocation[1]

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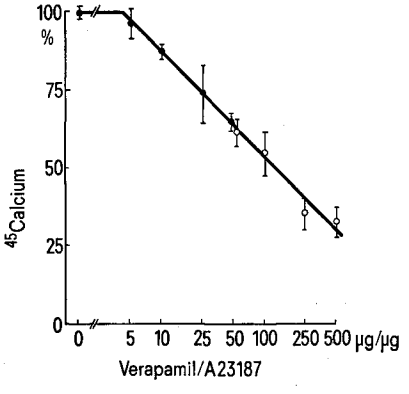
Summary. Verapamil and other organic calcium-antagonists inhibit the A23187-mediated translocation of calcium from an aqueous into an organic phase.

Organic calcium-antagonists such as verapamil and D600 are thought to inhibit the entry of calcium into cells across the plasma membrane, and are currently used to investigate the participation of such a calcium influx in calcium-dependent functional processes, e.g. contraction and secretion[2,3]. However, the precise mode of action of these drugs, at the physicochemical level, remained to be elucidated. It is here proposed that organic calcium-antagonists directly interfere with the calcium-binding sites of ionophores.



Effect of verapamil upon A23187-mediated calcium translocation. The amount of ^{45}calcium recovered in the immiscible phase is expressed in percent of the appropriate mean control value found in the absence of verapamil, and is shown as function of the ratio between verapamil and ionophore concentrations (µg/µg) in the initial organic phase. The experiments were performed in the presence of A23187 at concentrations of 20 (closed circles) and 2 (open circles) µM in the initial organic phase. Each point (± SEM) refers to 3 individual measurements. The control values for the concentration of calcium in the immiscible phase averaged 140.4 ± 2.0 and 2.2 ± 0.1 nM at ionophore 20 and 2 µM respectively, the experiments being carried out at a 11 µM concentration of calcium in the initial aqueous phase.

A small volume (0.2 ml) of Hepes buffer (25 mM; pH 7.0) containing Na^+ 123, K^+ 5 and Cl^- 115 mEq/l and ^{45}calcium (10 µCi/ml) was vigorously mixed for 1 min at room temperature with an equal volume of a mixture of toluene-butanol (7/3, v/v) containing, as required, the ionophore A23187 (Eli Lilly, Indianapolis) and various organic calcium-antagonists. The supernatant immiscible phase was then examined for its radioactive content.

The ionophore A23187 provokes a dose-related and reversible translocation of ^{45}calcium from the Hepes buffer into the immiscible phase. In addition to previous studies[4], extensive investigations on the modulation of ^{45}calcium translocation by temperature, mixing time, volume of reagents, pH, and concentration of ionophore, monovalent cations, calcium and other divalent cations established the validity of the present system as a model for the study of the ionophoretic properties of A23187 (unpublished observations).

As shown in the figure, verapamil inhibited the A23187-mediated translocation of calcium. The degree of inhibi-

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tion was proportional to the logarithm of the ratio of verapamil to ionophore concentration, each 10fold increase in such a ratio causing a $^1/_3$ reduction in calcium translocation. This relationship is comparable to that characterizing the inhibitory action of organic calcium-antagonists upon physiological processes such as glucose-induced insulin release, which is likewise reduced by 30–40% for each 10fold increase in drug concentration[5–7]. Incidentally, it was already shown that verapamil also inhibits the secretory response evoked by the ionophore A23187 in both the neurohypophysis[8] and pancreatic B-cell[9].

The inhibitory effect of verapamil upon A23187-mediated calcium translocation was invariably present, whatever the calcium concentration of the initial aqueous phase. However, when the latter concentration was raised and the verapamil/ionophore ratio kept constant, the organic antagonist caused a lesser reduction in the apparent amount of active ionophore molecules, as judged by reference to the normal dose-action relationship for calcium translocation at variable calcium and A23187 concentrations. Thus, in the present model, as in living cells[10], calcium itself protected in a competitive manner against the inhibitory effect of the organic calcium-antagonist. Such a protective effect of calcium indicates that verapamil acts at the calcium-binding site of A23187, rather than causing an unspecific and direct alteration of the ionophore molecule itself. Verapamil exerted no obvious effect upon calcium movements in the absence of ionophore. It did not abolish the reversibility of A23187-mediated calcium translocation and failed to affect the apparent positive cooperativity between calcium and the residual active ionophore molecules (data not shown). Essentially the same results were obtained when verapamil was added to the initial aqueous as distinct from organic phase. Comparable results were also obtained with other organic calcium-antagonists, including R33711[7] and suloctidil[11].

In conclusion, organic calcium-antagonists interfere, in an artificial model, with the ionophoretic property of A23187. It is postulated that a similar interference of these drugs with the calcium-binding sites of native ionophoretic systems located in the plasma membrane may account for their inhibitory action upon calcium handling in living cells.

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Synthesis and properties of praziquantel, a novel broad spectrum anthelmintic with excellent activity against Schistosomes and Cestodes

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Summary. Synthesis and properties of 2-cyclohexylcarbonyl-1,3,4,6,7,11b-hexahydro-2H-pyrazino [2,1-a] isoquinolin-4-one, a novel anthelmintic with excellent activity against all species of Schistosomes pathogenic to man and a wide range of cestodes, will be reported.

Although diseases caused by helmintic infections are of great importance in human and veterinary medicine, only few therapeutic agents are available for mass eradication programs[1]. Requirements for mass treatment include high efficacy against all stages and the different species of parasites, only a few doses per treatment, low toxicity and chemical stability. It has been estimated that some 200 million people are infected with schistosomes, but none of the presently available schistosomicides appears to meet all criteria desired for use in mass treatment programs[1,2]. About 100 million persons in the world are estimated to be infected with intestinal cestodes[3,4] and economic loss through cestode infections in animals is considerable. While satisfactory advances have been made in the treatment of many adult cestode infections, no drug is available which acts against all cestode species, that are of importance in human and veterinary medicine, and treatment of larval stages still presents an unsolved problem. In this note we want to report the discovery of Praziquantel[5–7], a highly promising broad spectrum anthelmintic for oral or parenteral use.

Praziquantel has been synthesized in E. Merck company and came out of an extensive investigation of the pharmaceutical potential of substituted isoquinolines derived from Reissert-compounds. Its anthelmintic activity was found by Bayer AG[7]. Praziquantel is 2-cyclohexylcarbonyl-1,3,4,6,7,11b-hexahydro-2H-pyrazino [2,1-a] isoquinolin-4-one (**2** for R = Cyclohexyl). From a large number of pyrazinoisoquinolinones (**2**), Praziquantel has been selected for further trials on grounds of its excellent therapeutic index[7,8] in experimental schistosomiasis and cestodiasis.