starfish arm nerve contains a relatively high concentration of T (1250 ng/g). The concentrations of PE or m-TA are low (< 5 ng/g) in the neural tissue of the species so far studied (table 3). The concentrations of the acid metabolites of DA or 5-HT in *Helix* ganglia are quite low (table 2); these findings are in very good agreement with low MAO activity[8-11] and with the low concentrations for DOPAC, HVA or 5-HIAA in the ganglia of *Helix* reported in an earlier investigation[14]. Claims that the ganglia of *Helix aspersa* contain large concentrations of DOPAC and HVA[23] were not supported by the present (table 2) or earlier experiments[14]. The most striking difference in the concentration of monoamines between the ganglia and foot muscle of *Helix* is the low content of p-OA in foot muscle (table 1); the concentration of the other amines or metabolites in the foot muscle are of about the same order (tables 1 and 2) as those in the ganglia.

These results support the suggestion that the trace amines may possess a neurotransmitter or neuromodulatory role in snail ganglia or foot muscle and further confirm that monoamine oxidase has a limited role in the inactivation of some of these amines in this species.

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Ionophoric activity of the antibiotic X.14547 A in the mitochondrial membrane

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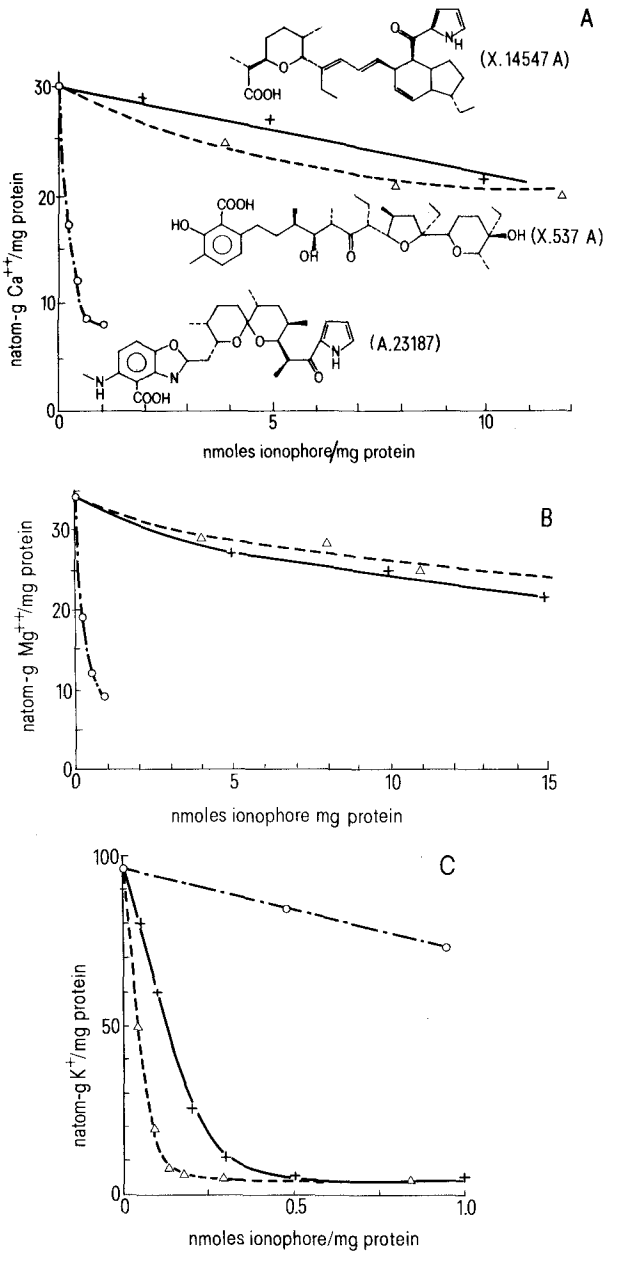
Summary. Release of Ca^{++}, Mg^{++} and K^+ by the carboxylic ionophore X-14547 A was studied in the mitochondrial membrane. A comparison was made with A.23187 (Calcimycin) and X.537 A (Lasalocid A) under the same experimental conditions. It was shown that in this test system X.14547 A is primarily a K^+ carrier comparable with X.537 A.

Antibiotics belonging to the large group of carboxylic polyethers generally act as monovalent cation carriers in membranes. However, a few are able to complex more specifically divalent cations and transport them through membrane phases; these make up a separate subgroup[1]. The anbitiotic X.14547 A, recently isolated from a strain of *Streptomyces antibioticus* NRRL 8167[2], is included in this subgroup as it carries Ca^{++} through a chloroform phase[3]. In methanol, the constants of formation of 2:1 neutral complexes (AH, A^- M^+ with alkali cations M^+ and 2 A^-, M^{++} with alkaline earth cations M^{++}) have been measured[4]. AH stands for the protonated form, A^- for the anion of the carboxylic antibiotic. X.14547 A has an unusual structure with a *trans*-butadienyl group connecting 2 heterocyclic systems as shown in figure A. Examination of models shows that it is not possible to obtain a folded structure forming a complexing cavity with a head-to-tail hydrogen bonding system as with A.23187[5] and X.537 A[6] which belong to the same subgroup and have molecular weights in the same range. This may explain the propensity of X.14547 A to form 2:1 complexes with M^+ in methanol as opposed to the others which give 1:1 associations[4]. Interestingly, the only crystalline structure available with this ionophore is also of the dimeric type AH, A^-M^+ where M^+ is replaced by the ammonium group of the chiral amine[7].

In a 3-phase system; aqueous phase/chloroform/aqueous phase the transport of calcium by X.14547 A is highly dependent on the pH of the compartment where the cation is extracted, the ionic flux being noticeable only for a pH above 8. This is dif-

ferent for A.23187 and X.537 A, where efficient transport lies in the pH range 7–8[4]. In addition X.14547 A is a carrier of K^+ and Mg^{++} in the same system (unpublished work). These results prompted us to study this ionophore in a relevant biological membrane. Such studies have been performed in rat liver mitochondria which remain a valuable test system for the newer ionophores[8]. A comparison was made with the ionophores A.23187 and X.537 A, under the same experimental conditions, by monitoring the release of the endogenous cations K^+, Mg^{++}, Ca^{++}.

Material and methods. Calcium transport. The experiments were carried out using 2 methods. In the first set of experiments, the mitochondria were added to the following medium: 250 mM Sucrose, 2 mM succinate-Tris pH 7.4–7.6, 0.04 mM $CaCl_2$. It was then possible to monitor the movement of Ca^{++} by an ion-selective electrode (Philips IS 561 Ca). The ionophore, dissolved in dimethyl sulfoxide, was added in every case after the uptake of Ca^{++} was near 40 natom-g./mg protein as indicated by the electrode response curve; an immediate efflux of Ca^{++} was then observed. The amount of ionophore added was decreased until a steady-state was established between the respiration-controlled uptake and the ionophore-induced release of Ca^{++}. With the same preparation of mitochondria, the following concentrations were necessary to obtain this steady-state: A.23187:0.08 nmol/mg protein, X.537 A:0.4 nmol/mg protein, X.14547 A:0.6 nmol/mg protein.



Effects of X.14547 A (+), X.537 A (△) and A.23187 (○) on the release of cations in mitochondria (A), Ca^{++}; B) Mg^{++}; C), K^+). Mitochondria (2 mg protein) suspended in 1 ml of medium (composition as indicated for the second set of experiments on Ca^{++} transport) were incubated 60 sec with the ionophore (in nmol/mg protein) then separated. The amount of cation was determined in natom-g per mg protein by atomic absorption.

In the second set of experiments, the mitochondria were incubated with the following medium: 200 mM Sucrose, 20 mM HEPES-NaOH (pH 7.4–7.6), 5 mM Glutamate-Tris, in the presence of increasing amounts of ionophore. Reaction was stopped within 60 sec by rapid centrifugation. Longer incubation was not necessary. The calcium content of the pellet was determined by atomic absorption. The endogenous Ca^{++} concentration is plotted versus ionophore concentration in figure A. Under these experimental conditions, without Ca^{++} added to the medium, the addition of 1 nmol A.23187 induced a release of 20 natom-g of Ca^{++}/mg of protein whereas 10 nmol of X.537 A or X.14547 A were necessary for the release of 8 natom-g of Ca^{++}/mg of protein. There is a strict similarity for the efficiency order: A.23187 $\gg$ X.537 A $\sim$ X.14547 A as tested by the 2 methods.

Magnesium transport. Results are plotted in figure B. The 3 ionophores induced a Mg^{++} efflux parallel to the Ca^+ efflux.

Potassium transport. As shown in figure C, A.23187 caused a small K^+ release. Previous careful studies in mitochondria for the same cation led to the conclusion that in the mitochondrial matrix[9,10] the K^+ movement was a secondary effect of the removal of regulating divalent cations. Our results are consistent with this interpretation.

For X.537 A or X.14547 A, less than 0.4 nmol/mg of protein induced an almost complete depletion of endogenous K^+ whereas amounts 30–50 times higher were necessary to observe a significant efflux of Ca^{++} or Mg^{++}. Many studies have been devoted to X.537 A, and although it has often been referred to as a calcium ionophore and indeed classified as a divalent polyether[1], it is well established that it can also carry monovalent cations and particularly that it acts as a H^+, K^+ exchanger in erythrocytes[8]. Several investigations have been carried out with X.14547 A. Liu et al.[3] showed a transport of both the monovalent cation Rb^+ and the divalent cation Ca^{++} with a 'Pressman cell using a chloroform phase. More recently, Bolte et al[4] with the same 3-phase system observed that X.537 A and X.14547 A were not efficient in the same pH range for Ca^{++} transport.

The present results do not conflict with these observations made with a simplified model. They provide new information concerning the cationic specificity in the mitochondrial membrane. In this test system, X.14547 A appeared principally as a K^+ ionophore like X.537 A, and the observed movements of Ca^{++} and Mg^{++} could be, at least partially, secondary effects due to the K^+ depletion in the mitochondrial matrix. Owing to the large difference in the chemical structure between these 2 ionophores, the similarities observed were not predictable. Duesler and Paul[6] mentioned that X.14547 A should have a strong tendency to form dimeric structures with cations. This is confirmed by measurements in methanol[4]. One may speculate that these dimeric species could participate in the mechanism of transport for K^+. Comparison of K^+ fluxes in bilayer membranes (BLM) with a monovalent polyether ionophore such as grisorixin, where the transporting species is a 1:1 neutral complex[11], should be worth investigation.

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Rabbit chondrocytes are binucleate in auricular but not articular cartilage[1]

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Ear cartilage chondrocytes have a single nucleus in the newborn rabbit but many become binucleate after 1 month of age[2–5]. Approximately 10% of articular chondrocytes in monolayer culture contain 2 nuclei[6]. It is not known whether this is true of the same cells in vivo. It has been reported that the mean DNA content of chondrocytes freshly isolated from the joints of rabbits is the same as that in culture[7]. If true, $^1/_{10}$ of the native cells may have more than 1 nucleus. The following study was undertaken to ascertain this point because aging of articular chondrocytes has often been proposed as the cellular basis of degenerative joint disease.

Materials and methods. Auricular and articular chondrocytes were isolated from 4 New Zealand White rabbits 0.3–4 years old (table 1). Absence of contaminating cell types following initial dissection was confirmed by histological examination of the cartilage. Chondrocytes were dissociated from the matrix by sequential digestion with hyaluronidase, trypsin and collagenase as described previously[8,9]. Because conventional smears of ear cells proved unsatisfactory, the chondrocytes were examined in histological sections. Articular chondrocytes were centrifuged into pellets directly and fixed in neutral buffered formalin. The procedure for auricular chondrocytes was modified slightly because their high fat content made them buoyant in the centrifuge. The cell suspensions were therefore first spun in Gey's balanced salt solution at 600 × g. The top 2 ml containing the floating cells were removed. Following aspiration of the remainder of the supernatant, the floaters were added to the cell pellet and the fluid made to 50% ethanol (v:v). This technical modification did not account for differences between the 2 cell types: an additional preparation of articular chondrocytes, employing the same ethanol-saline centrifugation step, yielded identical results. The suspension was recentrifuged and the resulting pellets processed as for the articular chondrocytes. They were embedded in JB4 glycomethacrylate (Polysciences, Warrington, PA), cut at a thickness of 2 µm, and stained with Giemsa[10]. The number of nuclei was counted in 400 cells of each group at a magnification of 1000 diameters. The dimensions of representative cells were measured with an eyepiece micrometer.

Results. In 3 rabbits 4–18 months old, 13.5–24.2% of auricular chondrocytes had 2 nuclei (table 1). 73% of these cells contained fat vacuoles. Double nuclei were seen in non-vacuolated as well as vacuolated cells (fig. A, B). The values presented may underestimate the actual proportion of binucleate auricular chondrocytes because the vacuolated cells were very large. The diameter was up to 29.8 µm while the length of the nuclei averaged 6.8 µm. An individual nucleus might thus not appear in a random section 2 µm thick. It is conceivable that false double nuclear profiles in auricular chondrocytes at times arose from bilobation or curvature of single nuclei. This is unlikely to account for the bulk of the binucleation observed because 1) auricular chondrocytes are binucleate and not bilobate in cell smear preparations; 2) the nuclei appeared discrete in adjacent sections of the same cell; and 3) articular chondrocytes, prepared by the same technique, appeared mononucleate.

By contrast, articular chondrocytes were not vacuolated. They were overwhelmingly mononucleate even up to 48 months of

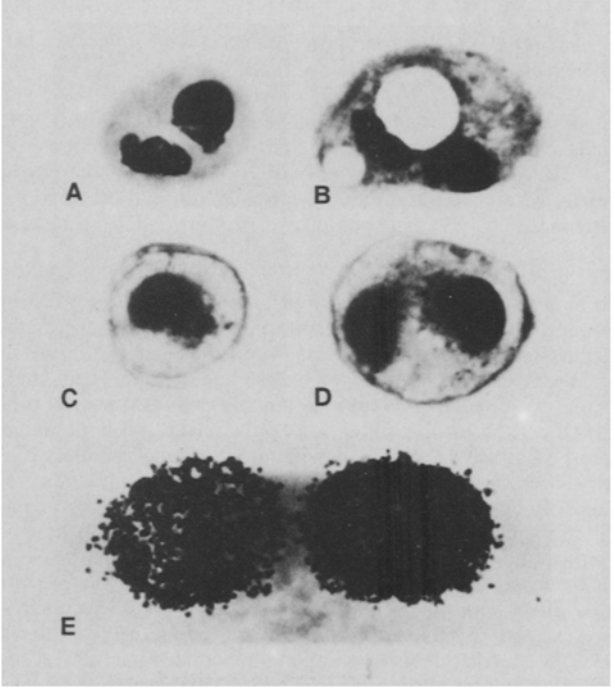

Figure 1. *A–D*, Sections of uncultured rabbit chondrocytes; *A*, binucleate ear chondrocyte that is not vacuolated; *B*, binucleate vacuolated ear chondrocyte; *C*, mononucleate articular chondrocyte; *D*, binucleate articular chondrocyte; *E*, Autoradiograph of ^{3}H-thymidine-labeled binucleate rabbit articular chondrocyte in monolayer culture. Giemsa, × 1500.