the respective diets with normal access to tap water. Each month, systolic blood pressure was monitored by the tail cuff method using a Narco electrosphygmomanometer. At 3-4 months of age, renal prostaglandin biosynthesis and catabolism was assayed by the radiotracer technique already published[2].

Results. While spontaneously hypertensive rats developed the usual sustained elevations in blood pressure when maintained on a normal diet containing 132 IU/kg of vitamin E, blood pressures were normal in those rats fed a diet deficient in this vitamin (12 IU/kg). The table shows blood pressure data for each of the groups at 3 and 4 months of age. Measurements of renal cortical prostaglandin catabolism during 3 separate studies on age-matched rats from each group show a highly significant elevation (2-3-fold) in enzyme activity only in the spontaneously hypertensive group fed the vitamin E deficient diet. A typical experiment is shown in the figure where the renal catabolic activity is compared between each of the 4 groups of rats fed a normal and a vitamin E deficient diet. Values observed for 15-hydroxyprostaglandin dehydrogenase catabolic enzyme were (mean ± SEM): normotensive, normal diet = 919 ± 75, deficient diet 922 ± 39; hyptertensive, normal diet 562 ± 18, deficient diet 1309 ± 220 ng product/10 min at 37 °C. Prostaglandin biosynthesis was not affected by the vitamin E deficient diet in either group.

These findings reveal that 1. expression of the hypertensive trait might be related to dietary factors, 2. reduction in dietary vitamin E intake appears to control the expression of this disease, 3. renal prostaglandin catabolism is enhanced in the experimental diet. Experiments are under way to determine if the observed reduction in blood pressures of the 'hypertensive' rats is permanent or will revert to the normally high levels when these rats are placed back on the normal (high vitamin E) diet.

Systolic blood pressures of normotensive (WKR) and hypertensive (SHR) rats maintained since birth on normal diet (ND, vitamin E = 132 IU/kg) and vitamin E deficient diet (DD, vitamin E = 12 IU/kg)

	Blood pressure (mm Hg ± SEM)	
	3 months	4 months
WKR		
ND	146 ± 3	146 ± 3
DD	129 ± 3	136 ± 3
SHR		
ND	173 ± 4	190 ± 2
DD	122 ± 4	127 ± 2

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The importance of the terminal hemi-acetal group for the ionophoric properties of nigericin

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Summary. Opening of the terminal hemi-acetal in dihydronigericin drastically reduces the ionophoric properties of nigericin and dehydroxymethylnigericin with 6 intact heterocycles. This is shown by 2 complementary methods, first with a liquid membrane electrode system, secondly by testing their ionophoric activities in rat liver mitochondria.

Carboxylic ionophores of the nigericin group are a large and growing class of antibiotics[2]. Solid state studies by X-ray diffraction showed that these polyethers adopt a pseudocyclic conformation with a head-to-tail hydrogen bond system, thus forming a cavity in which an unhydrated alkali cation can be complexed[3]. The resulting neutral complex has a strongly lipophilic outer envelope with a hydrophilic pole formed by the carboxylic group[4]. These amphiphilic characteristics can account for the well established ability of these molecules to transport cations through membranes[4].

In the course of our investigations concerning the complexing cavity formation, we studied nigericin[5] (**I**) (isolated from the strain NRRL B 1865) and 2 derivatives, dihydronigericin (**II**), and dehydroxymethylnigericin (**III**) first prepared by Chamberlin[6]. We tested their cation transporting abilities by 2 complementary methods.

1. The selectivity scales for alkali cations were measured with a liquid membrane electrode containing the ionophore as sensor in the membrane. EMF-values obtained are plotted against the reciprocal Goldschmidt ionic radius (figure 1). We have thus a measure of cationic affinities, relative to potassium, for the 3 compounds. For nigericin (**I**) we observed the order $K^+ > Rb^+ > Na^+$, Cs^+, Li^+ with a clear preference for K^+ and slighthy less for Rb^+, in good agreement with previous results[7]. The opening of the hemi-acetal F by $NaBH_4$ in **II** even though creating 2 hydroxyl groups, resulted in a derivative which had lost almost the whole discriminating power of the natural molecule. Furthermore, treatment of **II** by $NaIO_4$ induced the formation of **III** with a reconstituted hemi-acetal F′. The selectivity curve of a carboxylic ionophore was again obtained, in which the complexing ability of the ligand was just slightly lowered compared with that of nigericin.

2. We tested the ionophoric activities of the compounds in rat liver mitochondria. The experiments were carried out as follows: the mitochondria were first loaded with K^+ glutamate in the presence of valinomycin. Then, 90 sec after, K^+ and glutamate effluxes were monitored in the presence of **I** ~ **III** by 2 different methods. The kinetics obtained are plotted in figure 2. The addition of **I** and **III** induced a release of 120 natoms K^+/mg protein/30 sec (figure 2A), with no measurable difference between the 2 structures. Dihydronigericin (**II**) had no effect at this concentration. Figure 2B shows glutamate movements, measured under the same conditions as for K^+. Valinomycin induced uptake of 26 nmoles/mg protein/90 sec of (^{3}H)-glutamate; the addition of **I** and **III** resulted in an equivalent release of the

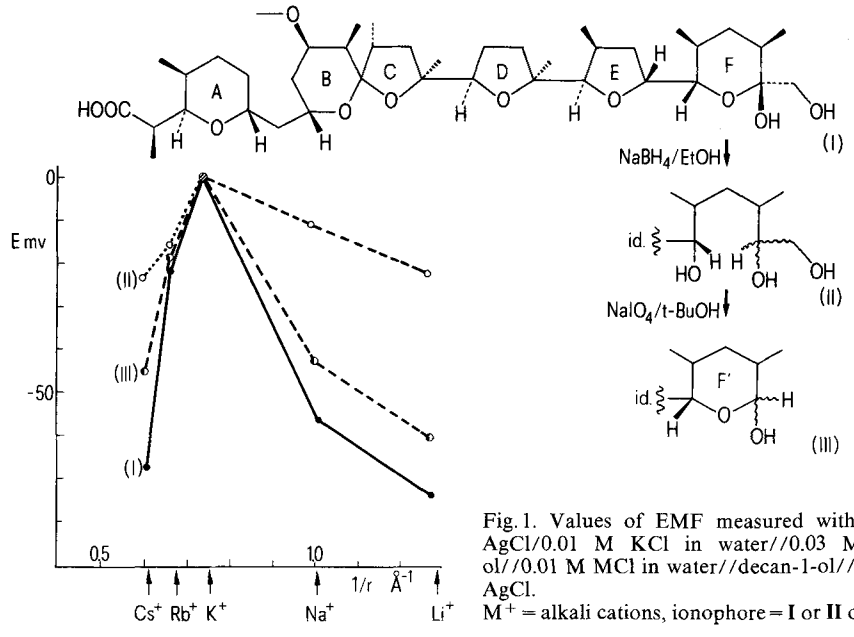

Fig. 1. Values of EMF measured with the galvanic cell[7,8]: Ag, AgCl/0.01 M KCl in water//0.03 M ionophore in decan-1-ol//0.01 M MCl in water//decan-1-ol//0.01 M KCl in water//Ag, AgCl.
M^+ = alkali cations, ionophore = **I** or **II** or **III**.

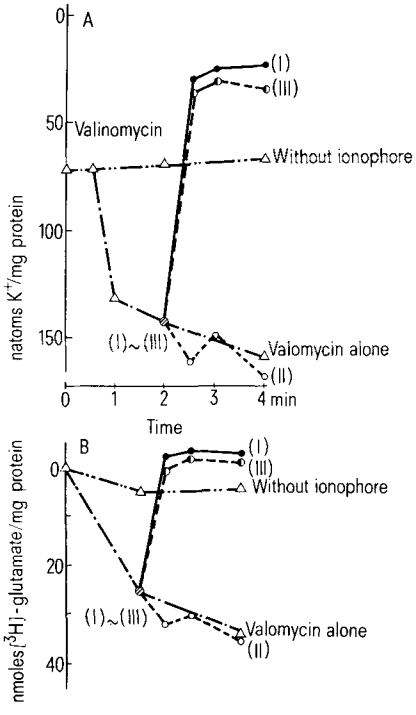

Fig. 2. *A* Effects of carboxylic ionophores **I** ~ **III** on K^+ efflux in rat liver mitochondria. The incubation medium was composed of 200 mM sucrose, 20 mM Hepes-KOH buffer pH 7, 5 mM $MgCl_2$, 5 mM glutamate-Tris. Mitochondria (2 mg protein) suspended in 1 ml of medium were incubated with valinomycin (50 ng/mg protein). After 90 sec compounds **I** ~ **III** (50 ng/mg protein) were added as indicated. Reaction was stopped within 30 sec at different times by rapid centrifugation and the potassium content of the pellet was determined in n atoms per mg protein by atomic absorption. *B* Effects of carboxylic ionophores **I** ~ **III** on (^{3}H)-glutamate efflux in rat liver mitochondria. The incubation medium was the same as in A, without glutamate. Mitochondria (2 mg protein) were preincubated in this medium (1 ml) with valinomycin (50 ng/mg protein). Glutamate transport was initiated by adding (^{3}H)-glutamate (5 mM final concentration) and stopped within 30 sec by rapid centrifugation at different times. The (^{3}H)-glutamate content of the pellet was determined in nmoles per mg protein. For further details on radioactivity measurements see Debise et al.[9]. As in A, **I** ~ **III** were added 90 sec after the zero time.

latter. **II** had no effect. Under our experimental conditions the glu/K^+ efflux ratio was close to 0.21 for **I** or **III**, which means that the K^+ efflux catalyzed by the ionophores causes a loss of negative charges as glutamate, with no stoichiometry between the 2 effluxes.

From the 2 sets of experiments the following conclusions can be drawn: a) there is a correlation between the cationic affinities obtained with the liquid membrane electrode and carrier properties as tested in a whole organelle like the mitochondrion.

b) these results demonstrate that the terminal hemiacetal F plays an important role in the transporting capability of the nigericin skeleton. It is thus likely that in a membrane the 6 heterocycles are maintained in defined orientations each relative to its neighbour, creating a cavity similar to that observed in the cristalline state[5,10] and also by NMR in solution[11]. A modification of this arrangement by opening 1 heterocycle, as in **II**, drastically decreases the ionophoric properties for structural and thermodynamic reasons, which we are now investigating.

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