The Effect of Valinomycin on Potassium and Sodium Permeability of HK and LK Sheep Red Cells

DANIEL C. TOSTESON, P. COOK, THOMAS ANDREOLI, and M. TIEFFENBERG

From the Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, North Carolina 27706

ABSTRACT A cyclic depsipeptide antibiotic, valinomycin, was found to produce increased selective permeability of the plasma membranes of HK and LK sheep red blood cells to potassium but not to sodium ions. The compound had relatively little effect on the active extrusion of sodium from HK sheep red blood cells or on the Na + K-stimulated ATPase activity of membranes derived from these cells. It is proposed that the selective cation permeability produced by this compound depends primarily on steric factors, particularly the relationship between the diameter of the ring and the effective diameter of the ion. The significance of these results for the problem of the mechanism of ionic selectivity in natural membranes is discussed.

INTRODUCTION

Valinomycin, a cyclic depsipeptide antibiotic, was first isolated from *Streptomyces fulvissimus* and characterized by Brockmann et al. (1, 2). Independently, MacDonald (3) studied the biosynthesis of the compound by a different organism (*Streptomyces* sp. (PRL 1642)). The original structure of the molecule proposed by Brockmann et al. was (—L-lactate—L-valine—D-hydroxyvalerate—D-valine—)$_n$ where $n = 2$. Subsequent work (4, 5) showed that this conclusion was erroneous and that n equals 3 in the naturally occurring antibiotic. It is noteworthy that all α-amino groups (of D- and L-valine residues) and α-hydroxyl groups (of L-lactate and D-hydroxyvalerate) in the molecule are involved in peptide or ester linkages respectively. The side chains of all residues consist entirely of one or more methyl groups. This cyclic structure is shown clearly in the space-filling Corey-Pauling model of valinomycin, a photograph of which is shown in Fig. 1.

The effect of valinomycin on mitochondria was first studied by McMurray and Begg (6) who showed that the compound under certain circumstances

both uncoupled respiration from phosphorylation and stimulated ATPase activity. Pressman (7, 8) showed that the stimulation of respiration by valinomycin required inorganic phosphate (Pi) and was associated with uptake of K^+ and extrusion of H^+ by mitochondria. Rb^+ and Cs^+ could replace K^+ in the system, but Na^+ and Li^+ could not.

Essentially similar results with valinomycin and mitochondria have been reported by Lynn and Brown (9), Graven et al. (10), and Chappell and Crofts (11). Shemyakin and his colleagues (12, 13) have synthesized and tested for antimicrobial activity a large number of analogues of valinomycin. Pressman (8) showed that relatively slight alterations in the structure of these

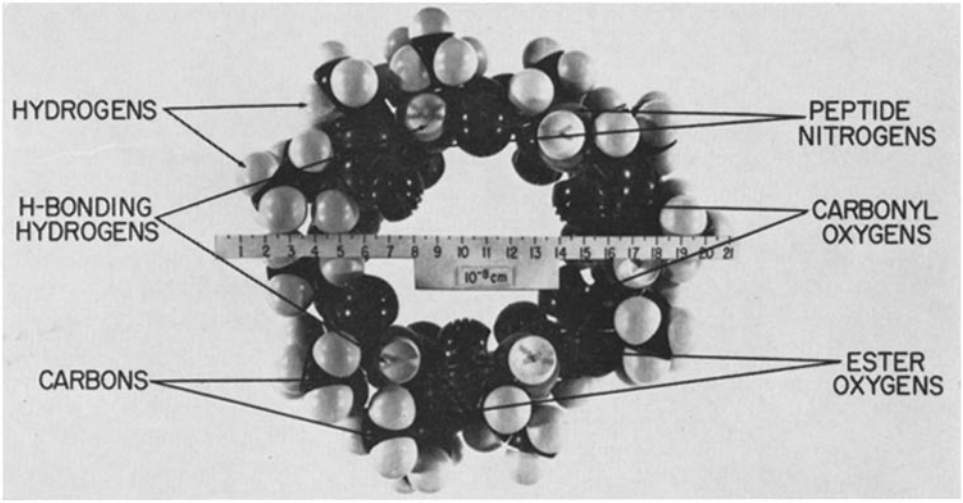

FIGURE 1. Corey-Pauling space-filling model of valinomycin.

synthetic valinomycin analogues (e.g., conversion of one D-valine residue to L-valine or one L-lactate to D-lactate) were sufficient to increase markedly the concentration of the compound required to produce a standard stimulation of respiration in the presence of K^+. More extensive alterations in the structure, e.g., conversion of all three D-valine residues to L-valine or reduction of n in the formula shown above from 3 to 2, resulted in apparent loss of the capacity to produce K^+-dependent stimulation of respiration.

Our interest in the effect of valinomycin on the ionic permeability of natural membranes arose from reports that this substance produced a specific permeability to K^+ in lecithin (14) and mixed lipid (15) bilayer membranes. A companion paper (16) reports our observations on the remarkable selectivities for alkali metal cations which valinomycin produces in thin artificial membranes formed from lipids extracted from high potassium (HK) and low

potassium (LK) sheep red cells. This paper describes the action of the antibiotic on the permeability of these two genetic types of red cells to K^+ and Na^+. The results with both intact red cell membranes and with thin lipid membranes indicate that valinomycin produces a marked increase in the passive permeability to K^+ but not Na^+. These results are consistent with the hypothesis that valinomycin acts as a molecular sieve or clathrate which admits the hydrated K^+ but not the Na^+ ion. This hypothesis is evaluated in relation to information presently available in the literature. The relevance of these reflections to the problem of the physicochemical mechanism of K^+-Na^+ selectivity in natural membranes is also considered.

METHODS

Measurement of Net Fluxes of K^+ and Na^+

Blood was drawn into heparin from HK and LK sheep by jugular venipuncture. The cells were washed three times in ice-cold NaCl (170 mM) and suspended (1 volume packed cells to 1000 volumes of medium, or about 3×10^7 cells per ml) in one of the following solutions. Low potassium medium consisted of NaCl (145 mM), KCl (5.0 mM), Na_2HPO_4 (9.35 mM), NaH_2PO_4 (1.65 mM) pH 7.4, glucose (11 mM). High potassium medium was identical except that the NaCl was replaced with KCl so that the KCl concentration was 150 mM. Valinomycin dissolved in ethanol was added directly to the cell suspension at the start of incubation. Control flasks received equal volumes of ethanol which contained no valinomycin.[1] Ouabain when used was added in aqueous solution. The cell suspensions were incubated at 37°C. Samples taken at the start of the incubation and at subsequent times were treated as described below.

An aliquot of the cell suspension was centrifuged and the separated cells washed four times with $MgCl_2$ (0.12 M). The washed cells were then hemolyzed with approximately 1000 volumes of a solution containing CsCl (0.004 M) and NH_4OH (1.25×10^{-3} v/v). Na and K concentrations in these hemolysates were measured directly with an atomic absorption spectrometer (Perkin-Elmer). Hemoglobin concentrations in the hemolysates were measured by the method of Drabkin (17). From these data and from the measured value of the mean hemoglobin content of the cells at the start of the experiment, the Na and K contents of the cells in each sample were computed. These contents are expressed in the tables in units of millimoles per that number of cells which occupied a volume of 1 liter at the start of the experiment. The actual concentration of K and Na in each sample of cells differs from the values which we computed because of changes in cell volume which occur during K loss or accumulation. Rate constants (see Table IV) for leakage of K and Na were calculated as previously described (18). Chloride ratio and cell volume values used in these calculations

[1] The valinomycin used in these studies was kindly given to us by Dr. T. C. MacDonald, Prairie Research Laboratories, Saskatoon, Saskatchewan, Canada. Preliminary pilot studies were carried out with valinomycin given to us by Dr. W. S. Lynn, Department of Biochemistry and Genetics, Duke University, Durham, North Carolina.

were computed from the assumptions of electroneutrality of cytoplasm and medium as well as osmotic equilibrium between these phases.

Unidirectional fluxes of Na were measured by procedures identical with those described above for net fluxes with the following modifications. HK cells were labeled with ^{22}Na by preincubation for 3 hr at 37°C in low K medium identical with that described above except that it contained a tracer amount of the isotope. The labeled cells were washed four times with about 10 volumes of ice-cold $MgCl_2$ (0.12 M) to free them of ^{22}Na present in the extracellular fluid. 1 volume of these cells was then suspended in 100 volumes of the following solutions: $MgCl_2$ (0.12 M), Tris-Cl (pH 7.4 at 37°C, 0.017 M) and KCl (either 10^{-5}, 5×10^{-4}, or 5×10^{-3} M), and incubated at 37°C for varying periods of time. Samples taken immediately after addition of the cells, 30, 90, and 180 min later, were treated essentially as described above for net

TABLE I

EFFECT OF DIFFERENT CONCENTRATIONS OF VALINOMYCIN ON Na AND K CONTENT OF HK SHEEP RED CELLS

This table shows the results of an experiment (No. 294) in which 1 vol of HK sheep red cells was incubated in 1000 vol. of either high K or low K medium fora period of 2 hr at 37°C. For details, see text.

			Original cation content retained					
			K			Na		
	Medium			Valinomycin			Valinomycin	
Time	K	Na	Control	10^{-7} M	10^{-6} M	Control	10^{-7} M	10^{-6} M
hr	*mM*	*mM*	%	%	%	%	%	%
0	5	165	100	78	42	100	88	84
2	5	165	106	60	0	90	85	100
0	150	20	100	100	97	100	100	104
2	150	20	98	112	128	82	85	85

fluxes. The medium and cells were analyzed for ^{22}Na by counting the annihilation radiation in an automatic well-type solid crystal scintillation spectrometer (Packard). The unidirectional fluxes were computed as previously described (18).

Adenosine triphosphatase (ATPase) activity was measured in HK sheep red cell membranes prepared by successive osmotic lysis as previously described (19). The rate of ATP hydrolysis was estimated from measurements of released inorganic phosphate by the method of Vreman and Jöbsis (20). The ionic conditions used in the assays are described in relation to the results.

RESULTS

The effect of two different concentrations of valinomycin on the sodium and potassium content of HK sheep red cells is shown in Table I. Note that 10^{-7} M valinomycin produced modest while 10^{-6} M valinomycin produced

marked reduction in the potassium content of these cells while the sodium content was changed relatively little. A more detailed study of the effect of 10^{-7} M valinomycin on the sodium and potassium content of HK sheep red cells is shown in Table II. In this experiment, HK sheep red cells were incubated in both a high and a low potassium medium. Valinomycin (10^{-7} M) produced marked movement of the potassium in the direction of decreasing electrochemical potential for the ion. By contrast, no appreciable net movement of sodium ions occurred despite the presence of substantial electrochemi-

TABLE II
EFFECT OF VALINOMYCIN ON Na AND K CONTENT OF HK SHEEP RED CELLS

This table shows the results of an experiment (No. 296) in which 1 vol of HK sheep red cells was incubated in 1000 vol of either high or low K medium for a period of 4 hr at 37°C. The relative volumes were calculated from the change in the Na + K content of the cells and the osmolality of the external medium (342 millimoles/liter) assuming isosmolality of cytoplasm and medium and that equimolar amounts of Na + K and Cl moved across the membrane. For details, see text.

	Medium				Cells		
					K	Na	$\frac{V}{V_0}$
					Millimoles		
Time	K	Na	Valinomycin	Ouabain	Original liter cells		
hr	*mM*	*mM*	*M*	*M*			
0	5	165	0	0	69	16	1.000
4	5	165	0	0	75	17	1.036
4	5	165	0	10^{-4}	70	17	1.036
4	5	165	10^{-7}	0	38	17	0.822
4	5	165	10^{-7}	10^{-4}	47	18	0.878
0	150	20	0	0	76	14	1.000
4	150	20	0	0	79	12	1.008
4	150	20	0	10^{-4}	77	13	1.006
4	150	20	10^{-7}	0	87	11	1.048
4	150	20	10^{-7}	10^{-4}	89	15	1.082

cal potential gradients for this ion, particularly in the case of cells suspended in a low potassium–high sodium medium. The rate of net loss or accumulation of potassium ions in the presence of valinomycin was not appreciably affected by the presence of ouabain (10^{-4} M). Changes in cell volume, computed from changes in the total cation content and the assumption of osmotic equilibrium, showed that the cells decreased in volume during K loss into a low potassium medium and increased in volume during K accumulation in a high potassium medium. Table III shows that essentially similar results were obtained in comparable experiments with LK sheep red cells. Net movements

of potassium but not of sodium in the direction of decreasing electrochemical potential occurred in the presence of valinomycin.

Table IV presents the rate constants for leakage of potassium (${}^{i}k_{K}^{L}$) into HK and LK sheep red cells in the absence and in the presence of valinomycin (10^{-7} M). These rate constants were calculated from the rate of net movement of potassium ions and the known electrochemical potential difference for K^+ in red cell cytoplasm as compared with the external medium (18). Net movements of potassium measured in the presence of ouabain (10^{-4} M) were used

TABLE III

EFFECT OF VALINOMYCIN ON Na AND K CONTENT OF LK SHEEP RED CELLS

This table shows the results of an experiment (No. 296) in which 1 vol of LK sheep red cells was incubated in 1000 vol of either high or low K medium for 2 or 4 hr at 37°C. Relative cell volume was calculated from the change in the total cation content of the cells and the osmolality of the external medium assuming isosmolality of cytoplasm and medium and that equimolar amounts of Na + K and Cl moved across the cell membrane. For details, see text.

	Medium				Cells		
					K	Na	$\frac{V}{V_0}$
					Millimoles		
Time	K	Na	Valinomycin	Ouabain	Original liter cells		
hr	*mM*	*mM*	M	M			
0	5	165	0	0	14	69	1.000
4	5	165	0	0	15	69	1.002
4	5	165	0	10^{-4}	13	69	0.992
4	5	165	10^{-7}	0	7.8	70	0.970
4	5	165	10^{-7}	10^{-4}	10.4	74	1.006
0	150	20	0	0	18	68	1.000
2	150	20	0	0	21	65	1.000
2	150	20	0	10^{-4}	20	66	1.000
2	150	20	10^{-7}	0	46	66	1.154
2	150	20	10^{-7}	10^{-4}	45	65	1.140

in the calculations in order to eliminate active transport as a mode of potassium transport. Because the magnitude of the net movements of potassium in the absence of valinomycin was very small in these experiments and, therefore, the computed values for the leakage rate constants unreliable, the control values in Table IV were taken from previous estimates (18). Note that valinomycin (10^{-7} M) produced a 10- to 20-fold increase in the rate constant for inward leakage of potassium in both HK and LK sheep red cells. The magnitude of the increase in ${}^{i}k_{K}^{L}$ in HK cells produced by valinomycin (10^{-7} M) was not altered by replacement of the Na^+ in the medium with Li^+, Cs^+, Mg^{++}, or Ca^{++}.

The potassium resistance (R_K) of the red cell membrane can be computed from the relation $R_K = \frac{RT}{F^2 M_K}$ where R is the gas constant, T is the absolute temperature, F is Faraday's constant, and M_K is K^+ flux when K^+ is distributed at equilibrium in the system (25). Although K^+ is not at equilibrium in the cells studied in these experiments, an approximate upper and lower

TABLE IV

RATE CONSTANTS FOR LEAKAGE OF K, HK AND LK SHEEP RED CELLS EFFECTS OF VALINOMYCIN

Rate constants for leakage of K were computed from measurements of net movements of K and Na in the presence of ouabain (10^{-4} M) and of valinomycin (10^{-7} M). Incubation conditions were as described for Tables II and III and in the text. The values presented are from two identical experiments (Nos. 295 and 296) in each of which there were duplicate control and experimental flasks. Control values in the absence of valinomycin were not significantly different from zero when estimated from these experiments. Therefore, control rate constants are taken from reference 18.

		k_K^i 10^{-3} (hr)$^{-1}$	
Cell type	Valinomycin	Experiment 295	Experiment 296
	M		
HK	0	(6.5)	
	10^{-7}	179	118
LK	0	(11.0)	
	10^{-7}	105	116

estimate of R_K can be obtained by using as M_K the leak efflux as compared with influx of K^+, calculated from the rate constants shown in Table IV and the known internal and external K concentrations. Such a calculation yields values of R_K between 10^6 and 10^7 ohm cm^2. These values are between 10^2 and 10^3 times higher than the resistance of thin lipid membranes in the presence of the same concentration of valinomycin and KCl (16). The source of this discrepancy is not clear. It could be due to a difference in the partition coefficient of valinomycin between aqueous solution and thin lipid membranes on the one hand, and aqueous solutions and intact sheep red cell membranes on the other. Experiments to test this point are in progress.

Table V shows that valinomycin (10^{-6} M) had no appreciable effect on the

sodium plus potassium stimulated–ATPase activity found in HK sheep red cell membranes. This was true both under optimal conditions for enzymatic activity ((Na) = 100 mM, (K) = 20 mM) and also when the concentration of potassium in the medium was substantially below that required to produce optimal activity. Similar results were obtained at even higher concentrations of valinomycin (10^{-5}) M).

TABLE V

Na PLUS K STIMULATED ATPase IN HK SHEEP RED CELL MEMBRANES. EFFECT OF VALINOMYCIN

This table shows the results of experiments in which the ATPase activity of sheep red cell membranes was measured in the presence and absence of valinomycin (10^{-6} M) in media containing different concentrations of K and Na. For details, see text.

Medium				ATPase activity	
Valinomycin	Ouabain	Na	K	$\frac{10^{-9} \text{ moles } P_i}{(\text{micromoles N}) \times \text{min}}$	$\frac{\text{Valinomycin}}{\text{Control}}$
M	*M*	*mM*	*mM*		
0	0	2	100	26	
0	0	100	0.0	24	
0	0	100	0.1	26	
0	0	100	1.0	38	
0	0	100	4.0	56	
0	0	100	20	54	
0	10^{-4}	100	20	24	
10^{-6}	0	2	100	25	1.0
10^{-6}	0	100	0.0	25	1.0
10^{-6}	0	100	0.1	26	1.0
10^{-6}	0	100	1.0	38	1.0
10^{-6}	0	100	4.0	56	1.0
10^{-6}	0	100	20	54	1.0
10^{-6}	10^{-4}	100	20	24	1.0

Table VI shows that valinomycin also had relatively little effect on the active extrusion of sodium from HK sheep red cells. In this experiment the ouabain-sensitive sodium efflux was measured at different concentrations of external potassium. The experiments were carried out in an external medium which contained almost no sodium in order to eliminate so-called sodium exchange diffusion (18). External sodium was replaced by magnesium ions. There was a slight but consistent reduction in the ouabain-sensitive sodium efflux in the presence of valinomycin (10^{-7} M). However, the magnitude of this effect was much smaller than the striking effect of valinomycin on the rate constant for leakage of potassium ions across the HK sheep red cell membrane.

DISCUSSION

The results reported above together with those described in the companion manuscript (16) clearly show that valinomycin produces an increased permeability to cations in both intact sheep red cell membranes and in thin artificial membranes prepared from lipids extracted from these cells. The increased cation permeability was observed directly as a net movement of potassium in the intact cells and indirectly as an increase in the conductance of thin lipid membranes. This increase in cation permeability is highly selective. Substantial movements of potassium occurred in both HK and LK sheep red cells in the absence of appreciable movement of sodium ions despite the presence of adequate driving forces for net passive movement of both ion species. Estimates of the ionic selectivity of thin lipid membranes based on measurements of biionic potentials indicated a sequence for monovalent

TABLE VI
THE EFFECT OF VALINOMYCIN ON ACTIVE SODIUM TRANSPORT IN HK SHEEP RED CELLS

The data shown in this table are taken from one of two identical experiments in which Na outflux from HK sheep red cells previously labeled with ^{22}Na was measured in the presence and absence of valinomycin (10^{-7} M) at different concentrations of potassium in the external medium. The flux values shown were computed from measurements made 1 and 3 hr after the start of incubation at 37°C. The concentrations of K and Na in the medium shown in the table are those actually measured at the end of 3 hr of incubation.

Medium				Cells		
				Na	Na outflux	
					Total	Pump
				Millimoles	10^{-4} moles	
Valinomycin	Ouabain	K	Na	Original liter cells	Original liter cells × hr	
M	M	10^{-4} M	10^{-4} M			
0	0	0.14	0.14	19	1.3	0
0	10^{-4}	0.18	0.16	18	1.3	
10^{-7}	0	0.54	0.10	19	1.4	0.6
10^{-7}	10^{-4}	0.66	0.09	18	0.8	
0	0	3.5	0.11	18	2.3	1.5
0	10^{-4}	3.5	0.10	19	0.8	
10^{-7}	0	4.0	0.13	18	1.8	1.0
10^{-7}	10^{-4}	3.9	0.09	19	0.8	
0	0	30	0.22	17	4.1	3.5
0	10^{-4}	32	0.10	19	0.6	
10^{-7}	0	31	0.20	17	3.7	3.0
10^{-7}	10^{-4}	31	0.12	19	0.7	

cations of $H^+ > Rb^+ > K^+ > Cs^+ > Na^+$. Despite these marked effects on the permeability of the red cell membrane to cations, valinomycin had relatively minor effects on the active extrusion of sodium from HK sheep red cells and on the sodium plus potassium–stimulated ATPase activity found in membranes prepared from these cells.

These results are consistent with the hypothesis that valinomycin increased the permeability of thin lipid membranes, both natural and artificial, by acting as a molecular sieve. The valinomycin molecule (see Fig. 1) is a cyclic depsipeptide in which all functional groups are involved in either ester or peptide linkage. Although careful titration curves of the compound have not, to our knowledge, been reported, it is highly unlikely that it is charged at neutral pH. Because of the large number of methyl groups in the valine and hydroxyvaline residues, the compound is almost insoluble in

TABLE VII

ESTIMATED DIAMETERS OF UNIVALENT CATIONS

This table shows values for ionic diameters taken from the literature (21, 22). The values designated as taken from reference 21 are from Table 17.

	Crystallographic (Pauling)	Stokes-Einstein (22)	Corrected hydrated (22)	Corrected hydrated (21)
Li	1.20	4.74	7.4	6.80
Na	1.94	3.66	6.5	5.52
K	2.66	2.50	5.0	4.62
Rb	2.96	2.36	4.7	4.56
Cs	3.38	2.38	4.8	4.56
$N(CH_3)_4^+$	6.94	4.08	6.94	—
$N(C_5H_{11})_4^+$	10.58	10.58	10.58	—

water. In water, the molecule would be expected to assume a conformation in which the carbonyl groups together with the ester oxygens and peptide nitrogens would be oriented toward the surrounding water molecules while the methyl residues would tend toward one another. Corey-Pauling space-filling models of the type shown in Fig. 1 can be rotated into a configuration in which most of the methyl groups are in the center of the molecule with the ester and peptide bonds oriented outward. In this case, the central hole is practically occluded. By contrast, when dissolved in nonpolar lipid, it would be expected that the open conformation of the molecule shown in Fig. 1 would be favored by hydrophobic interactions between the methyl side chains and the solvent. Under such circumstances, it seems likely that the ring would be filled by water molecules or other polar substances such as ions. According to this view, the carbonyl oxygens which form the perimeter of the central hole in valinomycin might substitute for water oxygens in the hydration shells of suitably sized ions.

A precise test of this hypothesis is difficult at present because of the lack of information about the exact size of the ring in the valinomycin molecule when it is located in thin lipid membranes and because of uncertainties as to the size of hydrated alkali metal cations. The effective diameter of the ring in valinomycin appears to be between 6 and 8 A when the molecule is in its most open conformation. Table VII summarizes estimates of the hydrated diameter of the alkali metal cations by two kinetic methods (21, 22). Despite considerable variations in the magnitude of these estimates of ionic diameters, note that the ionic diameter for hydrated sodium is consistently greater than that for hydrated potassium, rubidium, and cesium. It is reasonable to conclude that the ionic selectivity of valinomycin for potassium over sodium depends upon the greater ease with which hydrated potassium ions can fit into the ring. It is not yet clear whether similar spatial considerations can account for the differences in selectivity for cesium, potassium, and rubidium ions all of which have approximately the same hydrated diameter when estimated by most methods. The fact that other cyclic esters and depsipeptides with substantially fewer ring atoms (e.g., the enniatins with 18 and the nonactin series with 32 as compared with 36 for valinomycin) can also produce high selectivity for K^+ as compared with Na^+ (15), suggests that the carbonyl oxygens in such molecules can substitute even for the primary hydration shell of the alkali metal cations. A somewhat similar mechanism which involves a best fit of ions with 0, 1, or 2 hydration shells into polar pores between macromolecules has been proposed previously by Mullins (23) to account for ionic selectivity of nerve membranes.

Elucidation of the precise mechanism by which valinomycin and other cyclic depsipeptides and esters produce an increased and selective permeability of thin artificial and natural membranes to alkali metal cations will require further investigation. In particular, it is not yet clear whether the charge-carrying element in the membrane is the potassium ion moving through an intramolecular pore made up of several valinomycin molecules lined up normal to the plane of the membrane or whether it is a complex of K^+ and valinomycin. Furthermore, the detailed relations between ionic selectivity on the one hand, and ring diameter as well as the chemical properties of ring atoms and side chains on the other, remain unclear. Experiments directed toward clarification of both these matters are now in progress in this laboratory.

The discovery that a cyclic depsipeptide like valinomycin can produce a striking increase in the selective cation permeability of thin lipid membranes raises interesting new possibilities in membrane physiology and pharmacology. In particular, this antibiotic is constructed from units, amino acids such as valine and hydroxy acids such as lactate and hydroxyvalerate, which are likely to be present in the macromolecules found in biological membranes.

Thus, it is entirely possible that proteins containing macrocyclic configurations could play an important role in determining the ionic selectivity of plasma as well as of intracellular membranes. A particularly attractive feature of this possibility is that it would allow a sensible chemical basis for the known genetic control of membrane permeability to ions (18). Furthermore, the probable susceptibility of the ring diameter of such configurations to the ambient chemical conditions, e.g., polar vs. nonpolar environment, the presence of competitors for the ring etc., provides a mechanism for the known plasticity of ionic permeability of natural membranes. For example, large increases in the passive permeability of human red blood cell membranes to potassium but not sodium ions are known to occur in several abnormal metabolic states (23, 24). It is interesting to speculate whether the reversible formation of a macrocyclic ring compound similar to valinomycin is involved in this phenomenon. Finally, elucidation of the rules which govern the relationship between the size of the ring and other chemical properties of macrocyclic esters, depsipeptides, and peptides and the ionic selectivity of these compounds should eventually permit the rational design of drugs which modify selectively the ionic permeability of natural membranes.

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