KELVIN LECTURE

Across the Living Barrier

By David E. Fenton
DEPARTMENT OF CHEMISTRY, THE UNIVERSITY, SHEFFIELD, S3 7HF

The purpose of the Kelvin Lectureship of the British Association for the Advancement of Science is 'to convey in non-specialist language to intelligent people who are not experts' the subject area under discussion. In order to retain the essence of the lecture only key references are given as it is hoped that this article, closely based on the above lecture, might act as a preface to further study.

1 Introduction

The essential metals of the body may be considered in two categories; bulk metals and trace metals. The bulk metals (sodium, potassium, magnesium and calcium) constitute about 1% of human body weight, whereas the trace metals (manganese, iron, copper, cobalt, zinc, molybdenum, vanadium and chromium) represent less than 0.01% of the same. A 70 Kg man has approximately 170 g of potassium present in his body of which about 9 g are in the blood and 3 g in the tissue fluid; the same man requires only about 5 g of iron.

Paradoxically it is the trace metals, of transition group origin, that have been studied most during the recent upsurge of interest in the involvement of metal ions in biological processes. This is because, due to the absence of *d* orbital electrons and suitable spin states, the bulk metals have few spectroscopic properties that may be studied and so have remained 'out of sight, out of mind',[1] despite their essentiality. They have no unpaired electrons and so cannot be studied by magnetic measurements or electron spin resonance spectroscopy, but it is possible to study sodium complexes directly by ^{23}Na nuclear magnetic resonance spectroscopy. In general it is the simplicity of the alkali metal cations that makes them difficult to study. The alkali metals provide highly mobile, unipositive cations and usually form weak complexes with ligands having 'hard' donor atoms such as oxygen.[2] They are concerned in vivo with the maintenance of normal water balance and distribution, the conduction of nerve impulses, the maintenance of neuromuscular activity and potassium helps the heart relax between beats.[3] These roles are related to the ionic character and mobility of the cations. Sodium and potassium are also required to activate

[1] R. J. P. Williams, *Nature*, 1974, **248**, 302.
[2] R. J. P. Williams, *Quart. Rev.*, 1970, **24**, 331.
[3] P. B. Chock and E. O. Titus, *Progr. in Inorg. Chem.*, 1974, **16**, 287.

certain enzymes, but the mechanisms by which they activate enzyme catalysed reactions are not clearly defined.[4]

A closer look at one pair of bulk metals, the alkali metals sodium (Na) and potassium (K) reveals a pronounced difference in the location of these metals. Na^+ is the principal extra-cellular cation, and K^+ is the principal intra-cellular cation. The relative concentrations of these ions are well illustrated for mammalian blood cells where for the blood plasma the levels are 5 mM Kg^{-1} (K^+) and 143 mM Kg^{-1} (Na^+) compared with levels of 105 mM Kg^{-1} (K^+) and 10 mM Kg^{-1} (Na^+) for the red blood cells.[5] There is, therefore, a discriminatory mechanism which controls the selective uptake of K^+ in the cell from its bathing fluid.

The cell is surrounded by a membrane which separates its aqueous interior from the bathing fluids and selectively allows into the cell ions and nutrients, and allows out unwanted material, or material produced for use elsewhere. The membrane functions as a 'living barrier',[5] and it is in this barrier, it is believed, that the process of cationic discrimination occurs.

The membrane is about 70 Å thick and is composed, in general, of proteins and lipids. These may vary qualitatively from membrane to membrane, and although the building blocks for the membrane are known, the precise architecture is not known. Early work with electron micrographs suggested a trilamellar structure formed from two electron dense layers (protein) separated by an electron lucent layer (lipid).[6] This view persisted for many years and the lipid has been shown to exist as a bilayer with the polar head groups of the lipids bedding into the protein, and the long hydrophobic, hydrocarbon tails giving it a central zone of low dielectric constant. The alignment of these chains has been confirmed by *X*-ray crystallographic studies on the bilayer formed by 1,2-dilauroyl-(±)-phosphatidyl ethanolamine.[7]

A more recent approach views the membrane as consisting of a lipid bilayer in which globular proteins float 'as icebergs'. This model is called the Fluid Mosaic Model,[8] (see Figures 1 and 2).

To reach the inside of the cell the alkali metals must traverse this barrier and in so doing must convey their charge across a medium of low dielectric constant. This is an unfavoured step because of the high electrostatic energy required to transfer an ion from the high dielectric aqueous bathing fluid into the low dielectric hydrocarbon. In order to overcome this it has been proposed that the cations move across in association with organic molecules, either individually (a carrier), by relays of carriers, or by associated pores present in the membrane (see Figure 3). The carrier is postulated as encapsulating the cation and thus

[4] C. H. Suelter, *Science*, 1970, **168**, 789.
[5] R. Levin, 'The Living Barrier', Heinemann, London, 1969.
[6] R. Harrison and G. C. Lunt, 'Biological Membranes', Blackie, Glasgow and London, 1975.
[7] P. B. Hitchcock, R. Mason, K. M. Thomas, and G. Shipley, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 3036.
[8] S. J. Singer and G. L. Nicolson, *Science*, 1972, **175**, 720.

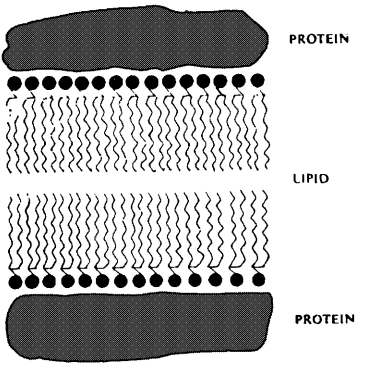


Figure 1 *The trilamellar structure of the membrane*

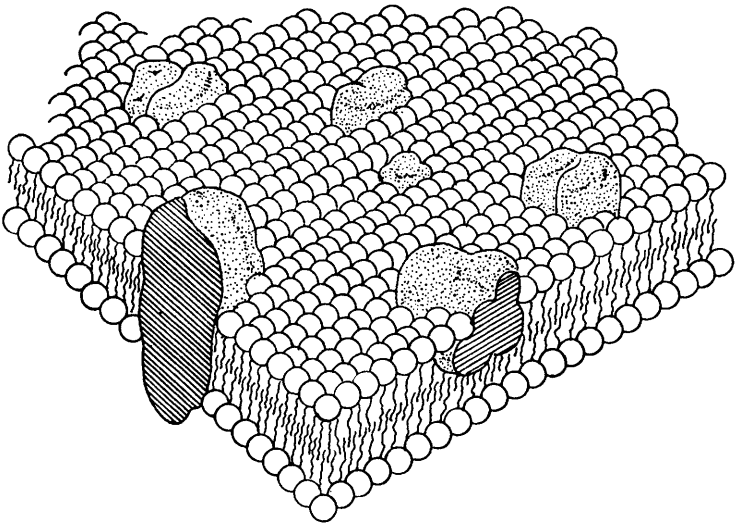


Figure 2 *The Fluid Mosaic Model for the membrane*
(Reproduced by permission from *Science*, 1972, **175**, 720)

effectively disguising it by presenting an organic, lipid soluble surface to the membrane. In this form the complex containing the alkali cation may cross the barrier either individually, by passing the cation from carrier to carrier, or by passing the cation from donor site to donor site in a pore. It is therefore necessary to know more about the nature of such systems, and their mode of operation.

This discussion is concerned with the carrier-assisted passive transfer of material independent of energy source—the so called facilitated diffusion. It is also possible for the cell to accumulate cations by working against concentration

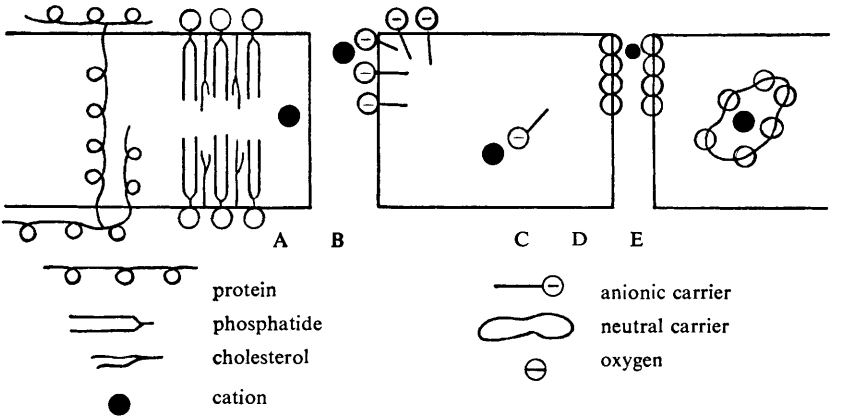


Figure 3 *Some modes of ion transport* (A, *Bare cation permeation;* B, *Negatively charged pore;* C, *Anionic carrier molecule;* D, *Neutral pore;* E, *Neutral carrier molecule*)

gradients. This process requires energy and is called active transport.[3,6] The hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphates is believed to provide the energy source for this process, generally known as the 'sodium pump'.

2 Macrocyclic Antibiotics and their Alkali Metal Complexes

In 1964, the American physiologist, B. C. Pressman found that certain antibiotics could induce the selective movement of K^+ into rat liver mitochondria.[9] These antibiotics, now termed collectively ionophoric (or ion-bearing) antibiotics could also increase the permeability of black-lipid films (synthetic lipid bilayers) to K^+. Such compounds are neutral at physiological pH and so may act as discriminatory cation carriers by forming complexes with the alkali metal cations. This discussion concerns these species although a further class of antibiotics containing carboxylic acid functions have also been found to exercise discriminatory powers.[10–13] This class can react with alkali metal cations to give 1:1 salts and so act as anionic carriers, or provide negatively charged pores.

Valinomycin is a cyclic dodecadepsipeptide, an atypical peptide having alternate amino- and hydroxy-carboxylic acids linked by amide and ester bonds, [L-valine-D-hydroxyisovaleric acid-D-valine-L-lactic acid]$_3$. It was first isolated from streptomyces sources and has been shown to have a high ion selective

[9] C. Moore and B. C. Pressman, *Biochem. Biophys. Res. Comm.*, 1964, **15**, 562.
[10] L. K. Steinrauf, M. Pinkerton, and J. W. Chamberlin, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 29.
[11] L. K. Steinrauf and M. Pinkerton, *J. Mol. Biol.*, 1970, **49**, 533.
[12] L. K. Steinrauf, E. W. Czerwinski, and M. Pinkerton, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 279.
[13] Yu. A. Ovchinnikov, V. T. Ivanov, and A. M. Shkrob, 'Membrane-Active Complexones' Elsevier, Amsterdam, 1974.

complexation of K^+ versus Na^+.[13] This selectivity has been retained, to a lesser extent, in numerous synthetic analogues of valinomycin. It was first proposed that the ion selectivity arose as a consequence of the size of the cavity in the antibiotic molecules as observed in models. The cavity was large enough to accommodate the hydrated alkali metal ion, however, *X*-ray structural determinations of the K^+-complex showed that it is the water free K^+ ion that is incorporated.

The conformation of uncomplexed valinomycin in *solution* was established by the composite use of ORD, CD, infra-red, ultraviolet and n.m.r. spectroscopic studies.[13,14] Valinomycin being in the form of a 36 membered ring presents numerous conformational possibilities and it is interesting to note that it is the first large peptide to have its structure established without recourse to *X*-ray techniques. The latter have, however, provided the final arbiter as subsequent *X*-ray studies, necessarily carried out in the *solid* state, on crystals recovered from different solvents and having different modifications, have shown quite different conformations from that proposed for valinomycin in solution.[15,16] The gross conformational features for the different crystalline modifications are nearly identical.

The conformation of valinomycin in solution has been found to be solvent dependent, and is an equilibrium of three major conformers (Figure 4). In

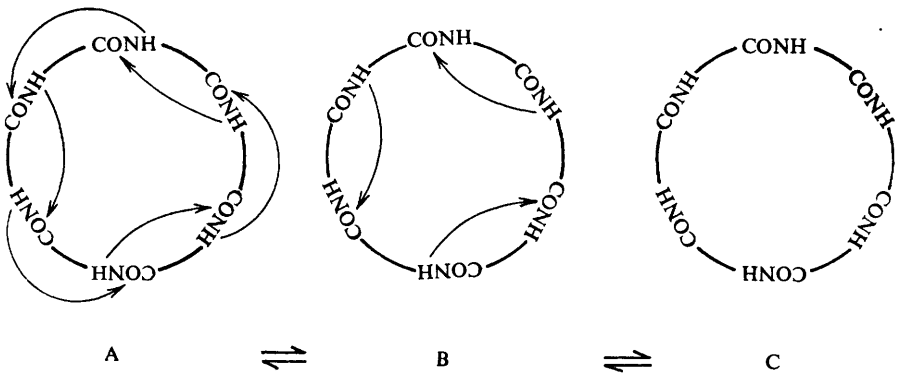


Figure 4 *The solution equilibrium of valinomycin*

solvents such as heptane or CCl_4 a bracelet-like structure is determined in which all NH groups are involved in hydrogen bonding (A). In polar solvents the hydrogen bonding breaks down such that only the D-valyl NH are involved, (B), and that at elevated temperatures and in highly polar solvents the NH

[14] D. J. Patel and A. E. Tonelli, *Biochemistry*, 1973, **12**, 486.
[15] G. D. Smith, W. L. Duax, D. A. Langs, G. T. de Titta, J. W. Edmonds, D. C. Rohrer, and C. M. Weeks, *J. Amer. Chem. Soc.*, 1975, **97**, 7242.
[16] I. L. Karle, *J. Amer. Chem. Soc.*, 1975, **97**, 4379.

groups are H-bonded to the solvent (C). The crystal structure[15] shows a different conformation (Figure 5) and although six H-bonds are maintained, only four are of the type present in the solution state, the remaining two being between ester carbonyls and NH groups. A possible mode of metal incorporation is

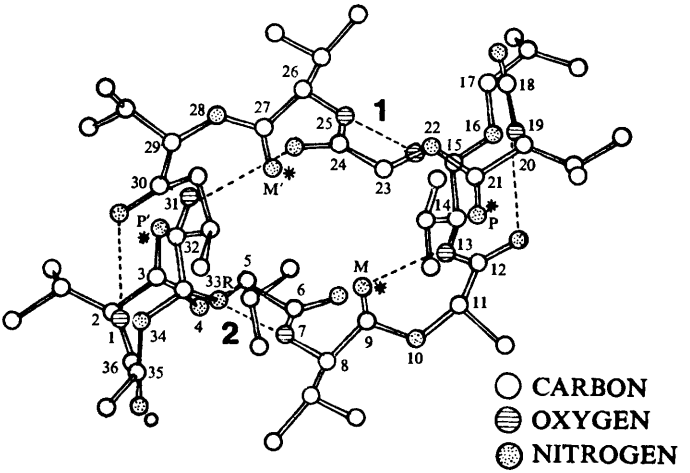


Figure 5 *The crystal structure of valinomycin*
(Reproduced by permission from *J. Amer. Chem. Soc.*, 1975, **97**, 7242)

suggested as two pairs of oxygen atoms lie in exposed positions on the molecular periphery (* in Figure 5). Either of these pairs could interact with an incoming K^+ ion, partially removing its hydration shell, and enticing it in to the hydrophilic interior. Stepwise removal of the hydration sheath could then occur as the sinuous ligand enfolded the metal.

The studies concerning the potassium complex have shown that it exists in essentially the same conformation in both solid and solution states (Figure 6)

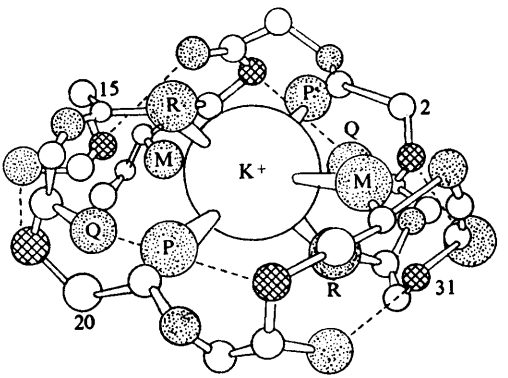


Figure 6 *The valinomycin–potassium complex*
(Reproduced by permission of Prof. W. L. Duax)

with inwardly orientated ester carbonyl binding to the metal.[13] The skeletal atoms wrap round the central cation as would three sine waves and are held in position by hydrogen bonds (Figure 7) which impart a rigidity to the molecular

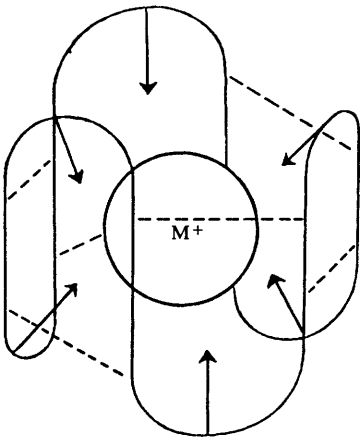


Figure 7 *Schematic diagram of a cyclododecapeptide folded to produce a cavity lined with six carbonyl donors (arrows) and stabilized by hydrogen-bonding (broken lines)*

frame. The metal cation interacts with the ester carbonyl oxygen atoms to give a distorted octahedral geometry, but the carbonyls do not point precisely at the metal cation to give maximum ion-dipolar interaction. The *X*-ray structural studies have been carried out on the $KAuCl_4$[17] and KI_3–KI_5[18] complexes. In solution the structure has been shown to be independent of the nature of the solvent.

The structure of the Na^+-complex, however, has been found to be solvent dependent[19], and the i.r. spectrum in solution shows an asymmetry of the ester carbonyl stretching frequency indicating non-equivalence of these carbonyls in the structure. The smaller size of Na^+ would lead to this observation as, if the cavity size is kept constant, it could not interact with all six carbonyl groups at once. This indicates that the availability of a precisely tailored cavity is essential to selectivity. When the H-bonded framework is destroyed by *N*-methylation of valinomycin selectivity is lost, again showing the necessity of retaining a precisely-tailored cavity for specific complexation.

Experiments with small bilayer phospholipid residues have led to the proposal that valinomycin is mainly embedded towards the inner, non-polar portion of

[17] M. Pinkerton, L. K. Steinrauı, and P. Dawkins, *Biochem. Biophys. Res. Comm.*, 1969, **35**, 512.
[18] K. Neupert-Laves and M. Dobler, *Helv. Chim. Acta*, 1975, **58**, 432.
[19] Yu. A. Ovchinnikov and V. T. Ivanov, *Tetrahedron*, 1975, **31**, 2177.

the bilayer.[20] Complex formation is believed to occur at the interface of the phospholipid bilayer and the aqueous phase. Precise details of the behaviour of valinomycin are, however, still unclear, some authors give preference to a single carrier model[21] whilst others refer to a relay mechanism.[22]

The ability of valinomycin and related compounds to act as potential carriers of alkali metal ions leads to the conjecture that similar species might exist in membranes. A cyclopeptide has been isolated from beef-heart mitochondrial membranes, and has shown transport properties[23], but the poor reproducibility so far obtained shows that more conclusive studies are required in this area.

A second group of antibiotics neutral at physiological pH and capable of alkali metal complexation is the enniatins.[13] They are isolated from fusarium sources and it is interesting to note that fusarium species have been shown to attack silicate minerals such as orthoclase releasing potassium.[24] These soil fungi not only liberate metallic ions from minerals, but can also provide antibiotics which will complex with alkali and alkaline earth metals.

Enniatin B is a cyclohexadepsipeptide, [D-hydroxyisovaleric acid-*N*-methyl-L-valine]$_3$, and the structure of the enniatin B–KI (Figure 8) complex resembles a

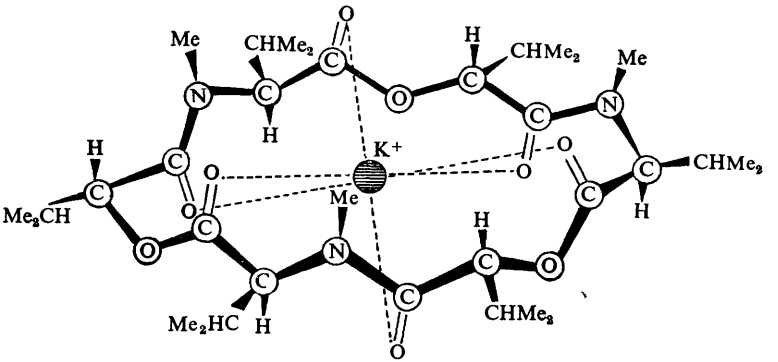


Figure 8 *The enniatin B–potassium complex*

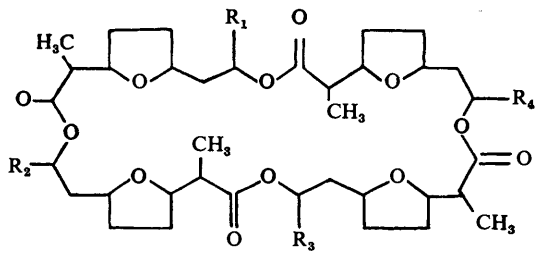
charged disc with lipophilic boundaries,[25] the metal being found at its centre. The molecules stack one above the other and if such a process should occur in a membrane then it is possible to envisage the formation of an ion-carrying pore. Solution studies on enniatins have shown the existence of 2:1 and 3:2 com-

[20] E. Grell, Th. Funck, and F. Eggers, in 'Molecular Mechanisms of Antibiotic Action on Protein Biosynthesis and Membranes', ed. E. Munoz, F. Garcia-Ferrandiz, and D. Vasquez, Elsevier, Amsterdam, 1972, p. 646.
[21] P. Läuger, *Science*, 1972, **178**, 24.
[22] Yu. A. Ovchinnikov, *F.E.B.S. Letters*, 1974, **44**, 1.
[23] G. A. Blondin, A. F. Decastro, and A. E. Senior, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 28.
[24] F. J. Stevenson, in 'Soil Biochemistry', ed A. D. McLaren and G. H. Peterson, Edwin Arnold Ltd., London, 1967, p. 139.
[25] M. Dobler, J. D. Dunitz, and J. Krajewski, *J. Mol. Biol.*, 1969, **42**, 603.

plexes, and taken to the limit it becomes quite possible to conceive of a pore mechanism for cation transfer using these antibiotics. There is also evidence from solution studies that in the 1:1 complexes the metal lies at the centre of the cavity but some authors doubt that this is so, especially as the conclusions of the *X*-ray analysis had not been carried through to the determination of atomic co-ordinates.[26]

There is a reduced K^+/Na^+ selectivity for the 1:1 complexes relative to valinomycin, which is ascribed to the greater flexibility of the latter; whereas the 2:1 complexes are reported to have a modest K^+ selectivity. In the latter the cation is effectively shielded from any interaction with its anion and so begins to resemble valinomycin more. Other naturally occurring cyclodepsipeptides such as the angolides, serratamolides and sporidesmolides which have features resembling enniatins have no reported alkali metal complexes.[13] Antamanide, a cyclodecapeptide isolated from *Amanita phalloides* mushrooms has a pronounced Na^+ selectivity but manifests little ionophoric activity.[27] The enniatins show reduced ionophoric activity relative to valinomycin, and this may be related to the incomplete encapsulation of the metal and so reduced lipophilicity of the complex.[13]

The third group of neutral ionophorous agents consists of the macrotetrolide actins which are isolable from actinomyces species.[13] Nonactin, so-named, it is said, because of its inactivity in early tests for biological activity, is depicted in Figure 9. It has a selectivity for K^+/Na^+ intermediate to valinomycin and enniatin B. The *X*-ray structure of free nonactin[28] shows a large cavity to be present and in the K^+-complex[29] this is occupied by the metal. The ligand wraps



$R_1 = R_2 = R_3 = R_4 = CH_3$	Nonactin
$R_1 = R_2 = R_3 = CH_3, R_4 = C_2H_5$	Monactin
$R_1 = R_3 = CH_3, R_2 = R_4 = C_2H_5$	Dinactin
$R_1 = CH_3, R_2 = R_3 = R_4 = C_2H_5$	Trinactin
$R_1 = R_2 = R_3 = R_4 = C_2H_5$	Tetranactin

Figure 9 *The actins*

[26] J. A. Hamilton, L. K. Steinrauf. and B. Braden, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 151.
[27] Th. Wieland, H. Faulstich, and W. Burgermeister, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 984.
[28] M. Dobler, *Helv. Chim. Acta*, 1972, **55**, 1371.
[29] M. Dobler, J. D. Dunitz, and B. T. Kilbourn, *Helv. Chim. Acta*, 1969, **52**, 2573.

itself round the metal as the seam of a tennis ball (Figure 10), with the four furanyl and four carbonyl oxygen atoms interacting with the metal. In the sodium complex a similar conformation is found but the metal–oxygen distances

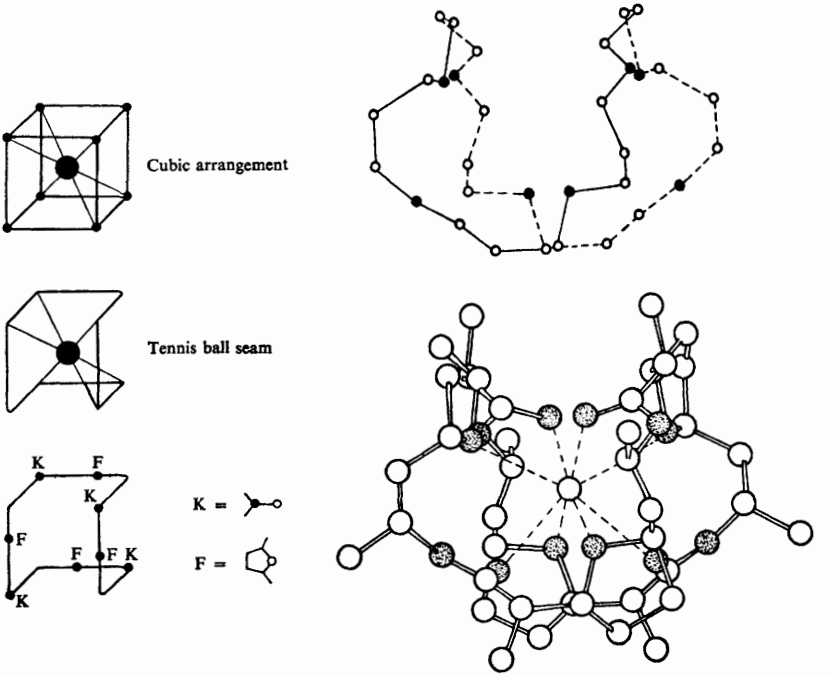


Figure 10 *The nonactin–potassium complex and the schematic representation of this molecule*
(Reproduced from B. T. Kilbourn, J. D. Dunitz, L. A. R. Pioda and W. Simon. *J. Mol. Biol.*, 1967, **30**, 559, and H. Diebler, M. Eigen, G. Ilgenfritz, G. Maass and R. Winkler. *Pure. Appl. Chem.*, 1969, **20**, 93, with permission)

differ showing a non-symmetric binding of Na^+;[30] K^+—0 (furan), 2.82 Å, K^+—0 (carbonyl), 2.77 Å, Na^+—0 (furan), 2.77 Å and Na^+—0 (carbonyl), 2.42 Å. This is a manifestation of the cavity–metal misfit due to the smaller ionic radius of the sodium ion. Similar observations have been made for alkali metal complexes of tetranactin.[31]

As with valinomycin it is possible to suggest a mode of incorporation of the metal ion.[28] Upon complexation the cation must be stripped of its hydration

[30] M. Dobler and R. P. Phizackerley, *Helv. Chim. Acta.*, 1974, **57**, 664.
[31] I. Sakamaki, Y. Jitaka, and Y. Nawata, *Acta Cryst.*, 1976, **B32**, 768.

shell, a process requiring loss of about 322 KJ mole^{-1} for K^+. This must be compensated for by interactions between the cation and the donor ligands in the complex. Complex formation has been shown to be very fast, and stepwise incorporation of the cation is essential.[32,33] Models show that a hydrated K^+-cation could be inserted into the nonactin cavity and held there by hydrogen bonding. Through a series of ligand conformational changes the water molecules could then be removed and the cation held in the cavity by ion–dipole interactions with the donor oxygen atoms of the ligand.

Certain common features may be found for the alkali metal complexes of the three groups of ionophores;

(1) the alkali metal sits in the ligand cavity at a centre of optimal electron density provided by the donor atoms of the ligand,

(2) a lipophilic exterior is presented to facilitate cation transport,

(3) a flexible ligand is required to effect the energetically favoured stepwise removal of the solvation sheath,

(4) a best-fit situation is required with regard to the cavity diameter and the diameter of the incoming ligand, but ligand–ligand repulsions must be minimized,

(5) the difference between the energy of ligation and energy of solvation must be maximized.

On fulfilment of these requirements the metal ion is given the appearance of a large organic moiety and so the lipid bilayer becomes the victim of a confidence trick in which it sees and transports an 'organic' cation across the barrier. It has been possible to provide evidence for both carrier and pore mechanisms for transport and investigations into the temperature dependence of ion selectivity have provided further insight into the problem. At 25 °C the selectivity ratio for K^+:Na^+ has been found to be of the order 10^4:1 whereas at 0 °C this ratio is reduced to only 2:1.[34] This dramatic diminishment has been interpreted as indirect evidence for a carrier mechanism as a pore mechanism would be unimpaired by freezing. In a carrier mechanism which necessarily involves a mobile ligand to effect incorporation and transfer of the metal freezing would cause loss of ligand mobility and so severely impair the mechanism. This would not be as serious in a pore. Other evidence to support this comes from experiments concerning the role antibiotics play in mediating the ionic conductance of lipid bilayers.[35] There is an abrupt loss of effectiveness in mediation for the presumed carriers nonactin and valinomycin occurring at the same temperature as the loss of membrane fluidity on cooling. This suggests that as in the selectivity experiments the mobile carriers are rendered inoperable on cooling. In contrast

[32] R. Winkler, *Structure and Bonding*, 1972, **10**, 1.
[33] M. Eigen and R. Winkler, in 'The Neurosciences: Second Study Program', ed. F. D. Schmitt, Rockefeller University Press, New York, 1970, p. 685.
[34] E. Eyal and G. A. Rechnitz, *Anal. Chem.*, 1971, **43**, 1090.
[35] S. Krasne, G. Eisenman, and G. Szabo, *Science*, 1971, **174**, 412.

the effects of an alleged pore-forming polypeptide, gramicidin, on mediation were unaffected by freezing. These observations do not preclude the presence of both mechanisms in a membrane as the alleged carriers were seen to act as carriers, and the alleged pore-former as a pore-former. If, however, gramicidin whose structure is proposed as a helix,[22] does form a pore, and if this is the route across the barrier, then it is plausible to suggest that in the fluid mosaic model where the protein striates the membrane, probably in helical array, the ionic selectivity and transport ability are inherent properties of the membrane components. This then alleviates the problem of a secondary system being present over and above the membrane's protein constituents.

For both the ionophorous antibiotics and the synthetic molecules which have been devised to mimic them a simple experiment may be carried out to illustrate both ion transport and ion selectivity.[36,37] A system is constructed, using a U-tube, consisting of two aqueous layers separated by a semipermeable medium, or membrane, ($CHCl_3$). Chloroform is chosen because its dielectric constant is similar to that for the membrane. A coloured alkali-metal salt such as potassium orthonitrophenolate is introduced on one side of the barrier and this is set aside as a control experiment [Figure 11(a)]. A second tube is then

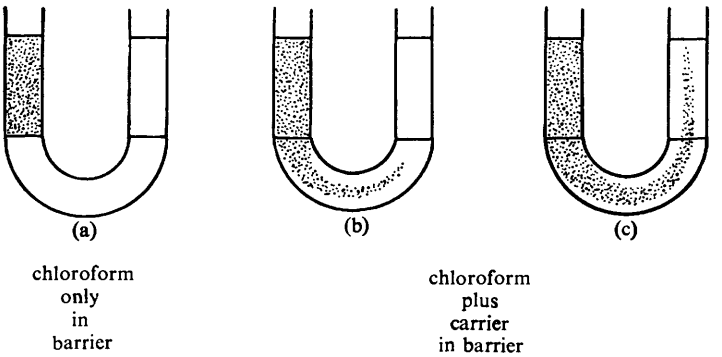


Figure 11 *The U-tube experiment: the shaded portions represent the colour imparted to the solvent phases by the potassium* o*-nitrophenolate; the right hand columns contain water*

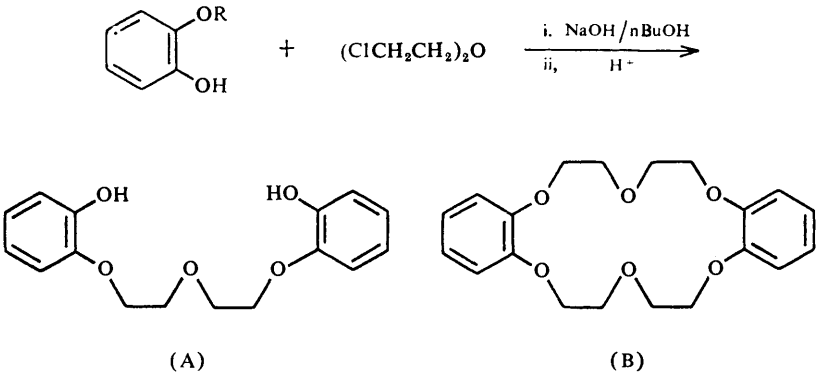
prepared, having a carrier species in the chloroform layer, and the movement of colour on transport of the metal salt by the carrier may be observed. If the colour transfers rapidly into the organic layer, but no further, the added molecule is acting as an ion receptor [Figure 11(b)]; if the colour is readily transferred through the $CHCl_3$ layer and on into the second aqueous layer then the added molecule is acting as an ion-carrier [Figure 11(c)]. The presence of K^+ in

[36] R. Ashton and L. K. Steinrauf, *J. Mol. Biol.*, 1970, **49**, 547.
[37] M. Kirch and J. M. Lehn, *Angew. Chem., Internat. Edn.*, 1975, **14**, 555.

either layer may be determined by spectrophotometric analysis (of the anion), or by atomic absorption spectrometry. The difference in behaviour is related to the stability constant of the K^+-carrier complex; too high a value leads to ion-reception, too low a value inhibits movement, and a middle range value leads to ion-carriage. This type of experiment may be carried out either qualitatively, or with a detailed analysis of transfer rates. A wide range of comparative results is obtained by changing the nature of the carrier or by altering the cation.

3 Cyclic Polyethers and their Alkali Metal Complexes

The rigours of obtaining macrocyclic antibiotics from fungal sources, or of synthesizing polypeptides, causes a requirement for more accessible probes for the processes of transfer. C. J. Pedersen, in 1967, reported the syntheses of a group of macrocyclic polyethers,[38] now colloquially termed crown polyethers, which have to an extent filled this role. Pedersen exploited an observation made during research into the development of ligands for use in the preparation of vanadium catalysts for the polymerization of olefins.[39] In attempts to synthesize the phenolic derivative (A), in the reaction scheme depicted, he isolated a fibrous white material (B), dibenzo-18-crown-6.



(A) (B)

(B) Arose from the reaction of residual unprotected catechol with the chloro ether. It was found to be insoluble in hydroxylic solvents, but, in the presence of added alkali a solubilization occurred. It was the recognition that this process was due to complexation of the metal by the polyether that opened up a new area of chemistry. Pedersen termed his compounds crown ethers because of their resemblance, in models, to royal crowns, and because of their ability to 'crown' alkali metal cations. The nomenclature adopted is as follows: dibenzo-

[38] (*a*) C. J. Pedersen, *J. Amer. Chem. Soc.*, 1967, **89**, 7017; (*b*) C. J. Pedersen and H. K. Frensdorff, *Angew. Chem. Internat. Edn.*, 1972, **11**, 16.
[39] C. J. Pedersen, *Aldrich Chim. Acta* (Aldrich Chem. Co., Milwaukee), 1971, **4**, 1.

describes the non-ethyleneoxy content, 18, the total number of atoms in the crown ring and 6, the number of heteroatoms in the ring.

A detailed study of the complexation properties of these polyether ligands led to the discovery of certain correlations.[38] Three types of complex were isolated; 1:1 (doughnut shaped), 2:1 (sandwich) and 3:2 (club sandwich), (Figure 12).

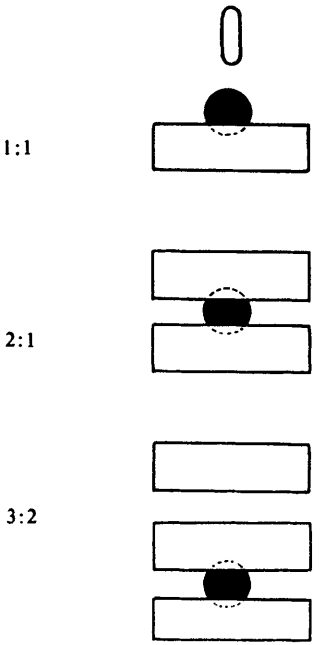


Figure 12 *Schematic representations for the predicted shapes of metal complexes o crown ethers*

The 3:2 complex has not yet been confirmed as existing in the *solid* state. A relationship between the ionic radii of the metals and the number of crown oxygen atoms was noted: Li^+ and 4 oxygens, Na^+ and 5 oxygens, K^+ and 6 oxygens, Cs^+ and 8 oxygens. This resembles the close-fit parameter for ionophore complexation, but did not prove absolute due to the flexibility of some ligands, and the general donor requirements of the metals. The stability constants[40] for the 5 oxygen crown complexes, (benzo-15-crown-5), with Na^+ and K^+ were similar and this was due to the stability of the 2:1 complex with K^+. The Na^+-cation fits into the 5 oxygen cavity but the K^+-cation is too large. However in order to sate its donor capacity a 2:1 sandwich complex is formed. A very strong complex was found for K^+ and the 10 oxygen crown (dibenzo-30-crown-10) and this was due to encapsulation of the metal by the crown ether, as occurs

[40] H. K. Frensdorff, *J. Amer. Chem. Soc.*, 1971, **93**, 600.

for valinomycin or nonactin. Several *X*-ray structural determinations are available for alkali metal complexes[41] of cyclic polyethers and representative structures are depicted below (Figure 13).

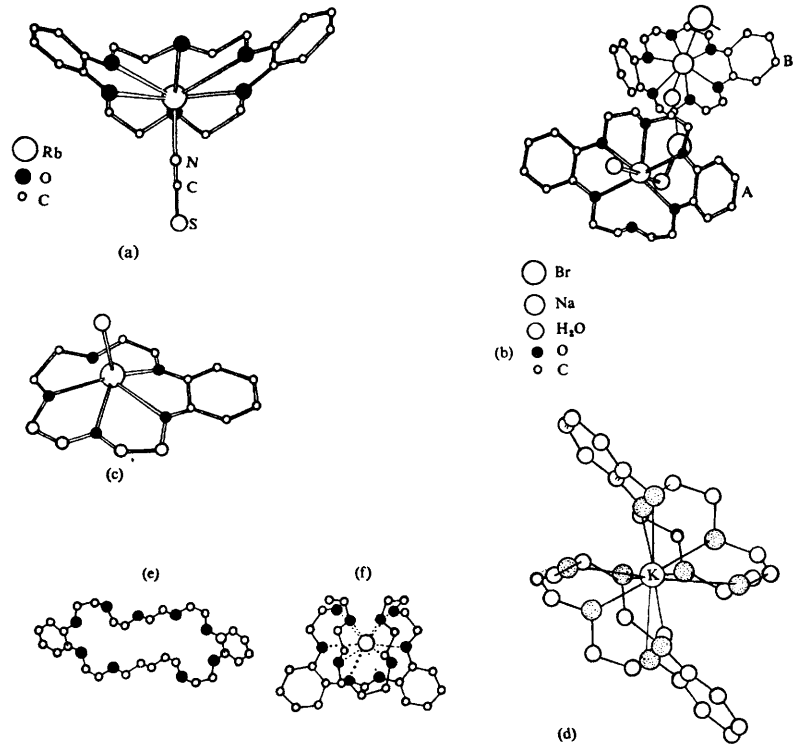


Figure 13 *Structures of some crown ether complexes:* (a), *Dibenzo*-18-*crown*-6,RbNCS *complex;* (b), *Dibenzo*-18-*crown*-6,NaBr,$2H_2O$ *complex;* (c), *Benzo*-15-*crown*-5,NaI,H_2O *complex;* (d), (*Benzo*-15-*crown*-5)$_2$,KI *complex;* (e), *Dibenzo*-30-*crown*-10; (f), *Dibenzo*-30-*crown*-10,KI *complex*
(Reproduced by permission from *Structure and Bonding*, 1973, **16**, 1; 1973, **16**, 71)

In dibenzo-18-crown-6,RbNCS, (a), the rubidium cation is co-ordinated to six coplanar oxygen atoms,[42] and to the nitrogen from the anion to give an ion pair. The rubidium cation lies slightly below the plane of the oxygen atoms, in the direction of the NCS^-, and the shape may be likened to that of an inverted umbrella. K^+ is slightly smaller than Rb^+ and it may be predicted that it would lie closer to the centre of the six oxygen atom torus. Superficially these 18-membered crowns may be likened to the enniatins which also have 18-membered

[41] M. R. Truter, *Structure and Bonding*, 1973, **16**, 71.
[42] D. Bright and M. R. Truter, *J. Chem. Soc.* (*B*), 1970, 1544.

rings, but whose lipophilicity is enhanced by the periferal groups making them more efficient operators in biological systems.[43]

The smaller cavity diameter in benzo-15-crown-5 leads, in general, to 1:1 complexation with Na^+ and 2:1 complexation with K^+. The structure of benzo-15-crown-5,NaI,H_2O,[44] (c), follows the pattern of (a); the sodium ion lies beneath the plane of the oxygen atoms but is here pulled towards the residual water molecule. The tenacity of water for sodium is further indicated in the complexes 18-crown-6,NaNCS,H_2O[45] and dibenzo-18-crown-6,NaBr,2H_2O, (b).[46] In (b) one molecule in the unit cell has sodium centrally disposed in the crown cavity with axial water ligands above and beneath it, whilst the second molecule has sodium pulled towards a bromide ion, the other axial site containing non-interacting water. The problem of removing water from the solvent shell before complexation can occur is well illustrated in the complex 12-crown-4,$Mg(H_2O)_6Cl_2$,[47] in which the structure contains octahedral $Mg(H_2O)_6^{2+}$ units, no dehydration having occurred, together with 12-crown-4 molecules hydrogen bonded to the complex cations. This again suggests a mode of incorporation of the crown, the first stage being hydrogen-bonding to the solvation shell followed by successive removal of the shell on conformational change of the ligand.

2:1 Complexes arise when a small cavity ligand is reacted with a large radius cation. $(Benzo\text{-}15\text{-}crown\text{-}5)_2KI$, (d), is a sandwich complex[48] as is $[(12\text{-}crown\text{-}4)_2Na^+]\,[Cl^-,5H_2O]$.[49] In the latter complex it is the halide anion that is hydrogen bonded to the water molecules, the Na^+-cation only interacting with the crown.

The macrocyclic dibenzo-30-crown-10 has perhaps the closest comparison with the ionophores as it resembles nonactin; the latter has a 32-membered ring and 8 metal–oxygen contacts in its complex. The structure of the free ligand (e)[50] has the form of a long closed loop, and on complexation of K^+ encapsulation occurs to give the 1:1 complex (f).[50] The visual resemblance with nonactin-K^+ is striking (see Figure 10), however, from solution data it has been shown that whilst both have the same selectivity pattern of $K^+ > Na^+$,[51] the dibenzo-30-crown-10–K^+ complex is stronger than the nonactin –K^+ complex ($\log K_{db} = 4.57$; $\log K_{non} = 3.58$ (MeOH solvent)). In biological experiments nonactin is more efficient than the polyether and this has been ascribed to the greater lipophilicity of the antibiotic. A better synthetic model is therefore required; reduction of the benzo-groups to cyclohexyl-groups, or the introduction of bulky side chains could help achieve this objective.

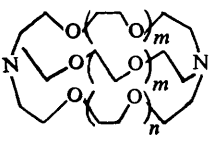
One stoicheometry observed for the crowns but not for the antibiotics, to

[43] D. C. Tosteson, *Fed. Proc.*, 1968, **27**, 1269.
[44] M. A. Bush and M. R. Truter, *J.C.S. Perkin II*, 1972, 341.
[45] J. D. Dunitz, M. Dobler, P. Seiler, and R. P. Phizackerley, *Acta Cryst.*, 1974, **B30**, 2733.
[46] M. A. Bush and M. R. Truter, *J. Chem. Soc.* (*B*), 1971, 1440.
[47] M. A. Neumann, E. C. Steiner, F. P. van Remoortre, and F. P. Boer, *Inorg. Chem.*, 1975, **14**, 735.
[48] P. R. Mallinson and M. R. Truter, *J.C.S. Perkin II*, 1972, 1818.
[49] F. P. van Remoortre and F. P. Boer, *Inorg. Chem.*, 1974, **13**, 2071.
[50] M. A. Bush and M. R. Truter, *J.C.S. Perkin II*, 1972, 345.
[51] P. B. Chock, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 1939.

date, is 1:2. Dibenzo-24-crown-8 has been found to encapsulate two K^+ ions[52] in the complex (dibenzo-24-crown-8), $(KNCS)_2$ and several other bimetallic complexes are now known.[53,54] The stoicheometry of one natural active transport system is that three sodium ions and two potassium ions move in opposite directions for every molecule of adenosine triphosphate hydrolysed and so the above observations indicate the possibility of a single carrier for more than one cation.

4 General Applications of Crown Ethers

Twenty years ago the alkali metals were 'out of sight, out of mind' to the co-ordination chemist. A prolific growth has followed the planting of seeds by physiologists ten years ago and now an extensive chemistry has developed which has spread to encompass the whole domain of the subject. Movement away from two-dimensional, monocyclic ligands to three-dimensional ligands such as the macroheterobicyclic 'cryptands'[55,56] (Figure 14) leads to the avail-



[1,1,1] $m = n = 0$
[2,1,1] $m = 0; n = 1$
[2,2,1] $m = 1; n = 0$
[2,2,2] $m = n = 1$
[3,2,2] $m = 1; n = 2$
[3,3,2] $m = 2; n = 1$
[3,3,3] $m = n = 2$

Figure 14 *Cryptands*

ability of rigid cages of finite diameter which give much more specific metal complexation than crown ethers.[57] This is because the tailoring of the cavity diameter to cation diameter is more precise. Cations of diameter larger than the optimal fit may be excluded from the cage, and smaller cations just rattle around forming only weak complexes. An extensive chemistry of this area has now been developed.

Crown ethers have also found application in organic synthesis.[58] On surrounding a cation with a crown ether solubility is conferred on the cation *via* hetero-atom solvation and accompanying enhancement of lipophilicity. The anion

[52] D. E. Fenton, M. Mercer, N. S. Poonia, and M. R. Truter, *J.C.S. Chem. Comm.*, 1972, 66.
[53] N. S. Poonia and M. R. Truter, *J.C.S. Dalton*, 1973, 2062.
[54] D. L. Hughes, *J.C.S. Dalton*, 1975, 2374.
[55] B. Dietrich, J. M. Lehn, and J. P. Sauvage, *Tetrahedron Letters*, 1969, 2885, 2889.
[56] J. M. Lehn, *Structure and Bonding*, 1973, **16**, 1.
[57] J. M. Lehn and J. P. Sauvage, *Chem. Comm.*, 1971, 440.
[58] G. W. Gokel and H. D. Durst, *Synthesis*, 1976, 168.

may be carried into solution at the same time, and so a previously organic insoluble base such as KOH may be rendered soluble (in aprotic media for example). This procedure is used effectively in phase transfer catalysis[59] where the reagent may be transferred from aqueous, or solid, phases into organic media by the formation of transient complexes. Homogeneous solutions of crown ether complexes have been used to study mechanisms, rates, and product distribution in nucleophilic substitution reactions in non-polar media.[58,60] Furthermore the movement from contact ion-pairs to solvent-separated ion-pairs allows for the provision of activated nucleophiles for synthetic purposes, *e.g.* KF releases F^-[61] and K(OAc) releases (OAc^-)[62], the so-called 'naked anions'.

The solubilization process is not restricted to salts, and alkali metals have been made soluble in THF, ethers, and amines, in the presence of crowns and cryptands.[63] One fascinating feature of this work has been the isolation and characterization of the crystalline species [cryptand 2,2,2]Na^+,Na^- as a stable compound.[64] Further applications have been made in inorganic chemistry, to the preparation of substituted phosphazenes and the stabilization of unusual anions. Hexachlorotriphosphazene reacts with KF, or KSCN, in the presence of 18-crown-6 to produce the corresponding hexafluoro-, or hexathiocyano-derivative[65]; and species such as Sn_9^{4-} and Pb_5^{2-} have been stabilized, and characterized by *X*-ray techniques using [2,2,2-cryptand]Na^+ as the counterion.[66] Cyclic polyethers have also been used in organometallic chemistry to investigate reaction pathways,[67] to prepare Group VI pentacarbonyl halides,[68] and as π-electron donors in complexes such as dibenzo-18-crown-6,$Cr(CO)_3$.[69]

The versatility of synthetic polyethers is further exemplified by their use in chiral recognition studies. Optically pure crowns have been found to complex optical isomers of amino acids, selectively effecting resolution.[70] This has led to their use as models for enzyme–substrate reactions, the area of 'host-guest',[70] or 'lock and key' chemistry,[71] discussed recently by Professor D. J. Cram in his 1976 Centenary Lecture.[72]

[59] J. Dockx, *Synthesis*, 1973, 441.
[60] D. J. Sam and H. E. Simmons, *J. Amer. Chem. Soc.*, 1972, **94**, 4024.
[61] C. L. Liotta and H. P. Harris, *J. Amer. Chem. Soc.*, 1974, **96**, 2250.
[62] C. L. Liotta, H. P. Harris, M. McDermott, T. Gonzalez, and K. Smith, *Tetrahedron Letters*, 1974, 2417.
[63] J. L. Dye, M. G. de Backer, and V. A. Nicely, *J. Amer. Chem. Soc.*, 1970, **92**, 5226.
[64] F. J. Tehan, B. L. Barnett, and J. L. Dye, *J. Amer. Chem. Soc.*, 1974, **96**, 7203.
[65] E. J. Walsh, E. Derby, and J. Smegal, *Inorg. Chim. Acta*, 1976, **16**, L9.
[66] J. D. Corbett and P. A. Edwards, *J.C.S. Chem. Comm.*, 1975, 984.
[67] J. P. Collman, J. N. Cawse, and J. I. Braumann, *J. Amer. Chem. Soc.*, 1972, **94**, 5905; J. P. Collman and S. R. Winter, *J. Amer. Chem. Soc.*, 1973, **95**, 4089.
[68] J. L. Cihonski and R. A. Levenson, *Inorg. Chem.*, 1975, **14**, 1717.
[69] K. H. Pannell, D. C. Hambrick, and G. S. Lewandos, *J. Organometallic Chem.*, 1975, **99**, C21.
[70] D. J. Cram, R. C. Helgeson, L. R. Sousa, J. M. Timko, M. Newcomb, P. Moreau, F. de Jong, G. W. Gokel, D. H. Hoffman, L. A. Domeier, S. C. Peacock, K. Madan, and L. Kaplan, *Pure Appl. Chem.*, 1975, **43**, 327.
[71] W. D. Curtis, D. A. Laidler, J. F. Stoddart, and G. H. Jones, *J.C.S. Perkin I*, 1977, 1756.
[72] D. J. Cram, 1976 Centenary Lecture, Chemical Society.

Despite the seemingly large amount of activity, this field of chemistry is still in a growth phase and some areas remain relatively unexplored. It is apparent that serendipity has played an important role in the development of models for antibiotics and that the spin-off areas, away from the immediate biological context of this report are rich in unmined chemistry. The interaction of alkali metal cations with acyclic species, polyethylene oxides[73] and linear polypeptides may yield useful information pertaining to the interaction of such cations with the protein in the fluid mosaic model of the membrane. Certainly complexes, albeit weak, are formed with those systems. There remains much more to observe and discover, and many results and applications to harvest from this field of study.

[73] D. E. Fenton, J. M. Parker, and P. W. Wright, *Polymer*, 1973, **14**, 589.