‘Don’t talk to me about permeability’

The Tenth Marjory Stephenson Memorial Lecture

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MARJORY STEPHENSON was the person mainly responsible for the early development of chemical microbiology in this country, was instrumental in starting this Society, served as its President, and was one of the first two women to be elected Fellows of the Royal Society. Your present President (Professor S. R. Elsdon) and I received our microbiological training from her hands and she infected us with her tremendous enthusiasm for, and enjoyment of the activities of, micro-organisms. I would like to thank the Society for giving me this opportunity to pay tribute to her in this the Tenth Marjory Stephenson Memorial Lecture.

Marjory Stephenson, or ‘MS’ as she was universally known, joined the Biochemistry Department at Cambridge in 1919 after the end of the First World War, which she had spent serving with the British Red Cross in France and Salonika. Encouraged by Professor Frederick Gowland Hopkins, she took up the study of bacterial metabolism, and her first paper, ‘Studies in the fat metabolism of the Timothy Grass *Bacillus*’, was published in 1922. In 1930 she published her monograph and, at one and the same time, established Bacterial Metabolism as a branch of experimental science, and herself as its outstanding authority. The book attracted many workers to the new subject, and knowledge of microbiology began to grow so rapidly that new editions of the book became necessary every few years. She began to prepare the second edition in 1936 and sought for someone to help in the laboratory while she engaged in the reading, abstracting and writing involved. I happened to be wandering around Cambridge, looking for some job where I could use my newly acquired knowledge, was offered, and happily, though somewhat fortuitously, grasped the opportunity to work with her.

The Biochemistry Department in Cambridge was an exciting place to work in at that time. Biochemistry was entering an exponential phase of growth: enzymes were being furiously separated and purified, coenzymes were being found and related to vitamins and growth factors, metabolic cycles were suspected and were being studied with tissue slices and cell suspensions rocked in Barcroft or Warburg manometers – but biosynthetic processes were divinely mysterious and bacteria seemed to be some form of separate creation. MS and her gang were showing that bacteria, apart from attacking such ridiculous substrates as carbon monoxide, phenol and methane, were curiously variable and could change their enzymic activities according to the conditions under which they were grown. We talked of ‘constitutive’ and ‘adaptive’ enzymes, the ‘glucose effect’, and variations with ‘age of culture’ which sooner or later trapped everyone. It all seemed rather unphysiological, especially to eukaryotes, and J. B. S. Haldane accused us of studying bacterial pathology. MS was convinced that human cells were capable of adaptation to unusual

substrates and spent some time investigating the levels of alcohol dehydrogenase and other enzymes in rats hooked on alcohol or morphine.

As I recollect, the first floor of the building housed MS, D. D. Woods and C. E. Clifton in one room, your last two Presidents shared another, while the Piries, D. E. Green, M. Dixon, R. L. M. Synge, R. Hill and W. E. van Heyningen were inhabitants of other rooms along the corridor. The stairwell housed a cold room, where it was rumoured that Bill Pirie once isolated a crystalline protein that turned to water when filtered at room temperature, and two 'large' centrifuges (taking all of 200 ml.) moored to yule logs to prevent them wandering down the stairs in an unbalanced moment. On these we prepared our washed suspensions of bacteria, and woe betide anyone who left wet tubes or broken glass around. The media room, some 10 × 4 ft, was in the hands of a formidable Charles, who might – or, if you were a research student, might not – prepare and sterilize the peculiar broths that so aroused the derision of Sir Paul Fildes, then engaged in the perfection of synthetic media for all sorts of bacteria. Instruction in bacterial metabolism came as a course of lectures to the part II biochemistry class given by MS, but, revere her as we may, no one could call her a brilliant lecturer. The lectures might well begin in the middle and end at the beginning but at least we gathered that someone had done something terrific. I forget most of the lectures I sat through in 1935–6 but I remember the subjects of MS's: Mueller's production of a nutrient medium for *Corynebacterium diphtheriae*; Stickland's reaction between amino acids in clostridia; Virtanen's work on nitrogen fixation; formic hydrogenlyase and hydrogenase; the oddities of autotrophic bacteria. At least you went away and read all about it.

MS was forthright and believed in striking while the temper was hot. There were days when we tiptoed round the lab, hoping that lightning really did not strike twice in the same place. Then the storm would pass, enthusiasm bubbled out of her room and we all joined in argument and riotous assembly while MS's laughter rang through the building. She believed that research meant taking up a challenge. In 1940–2 I worked on amino acid decarboxylases of bacteria, found that amines were produced only under acid conditions and wondered whether they were broken down under alkaline conditions. So I looked into the metabolism of amines by bacteria and eventually wrote what I thought was a paper 'rounding up' the story. MS read it through and judged: 'This is a pot-boiler, Ernest; get your teeth into something more difficult.' You knew where you were with MS. I often wonder what would happen if MS were a member of some of our student-staff committees these days.

When she had an idea she acted on it forthwith. At the time of the abdication of Edward VIII, while the rest of us were holding indignation meetings of one sort or another, MS was down in the telephone booth dictating a telegram of support to Buckingham Palace. In the same way she had little sympathy with theory unless it was backed by solid experimental evidence and she strongly supported the definition of science as knowledge based on observation and experiment. In the introduction to the second edition of her book in 1938 she wrote, of bacterial growth, 'Happily this subject now attracts mathematicians and statisticians less than formerly but has passed into the hands of biochemists...' In a review of enzyme variation in bacteria, I wrote, in 1943, 'If the rate of breakdown of a substrate is limited by the rate of diffusion of that substrate through the cell membrane, then it follows that apparent variations in enzyme activity may be due to alterations in the permeability of the membrane,' and proceeded to discuss the nature of the two forms of *Escherichia coli mutabile*. This was a non-lactose-fermenting strain of *E. coli* which, when grown for long periods on solid medium containing lactose, developed small pimples of

lactose-fermenting cells sitting on the main non-fermenting colonies. Deere, Dulaney & Michelson (1939) had shown that the non-fermenting organisms were able to ferment lactose if they were first treated with acetone or other membrane damaging substances. It was at a discussion of this situation (and my review of it) that MS exclaimed, 'Don't talk to me about permeability – that is the last resort of the biochemist who cannot find any better explanation.'

Shortly after that we discovered the existence of high concentrations of free amino acids inside bacterial and yeast cells (Gale, 1947; Taylor, 1947) and an experimental attack on the passage of certain amino acids into and out of the cell became possible. The mechanism of this transport, often against a concentration gradient of several orders of magnitude, has remained a subject of interest and importance and, *pace* MS, I propose to talk about permeability and to illustrate more recent developments by reference particularly to the transport of amino acids across bacterial membranes.

It was at the first symposium of this Society to be held after the death of MS that Dawson (1949) showed that the outside envelope of the bacteria cell could be removed, and so made possible a direct attack on the bacterial surface. In specific cases the outer wall could be digested and dissolved by the action of lysozyme (Salton, 1952) and removal of the cell wall in this way from *Micrococcus lysodeikticus* or *Bacillus megaterium* left an osmotically sensitive, membrane-bounded protoplast (Tomcsik & Guex-Holzer, 1952; Weibull, 1953). I and my colleagues had already found that amino acids such as glutamate, aspartate lysine, etc., were taken up by cells such as *Staphylococcus aureus* and accumulated in the free state within the cell so that the internal concentration could be two or three orders of magnitude higher than that in the external medium. Mitchell & Moyle (1956) showed that the volume of *S. aureus* impenetrable to glutamate (in the absence of a source of energy) equalled that of the protoplast and that it was the protoplast membrane that imposed an osmotic barrier between internal and external media. With few exceptions the passage of amino acids into the cell against this concentration gradient proved to be an energy-requiring process and, in due course, it was shown that specific transport mechanisms were involved, were under genetic control and gave an evolutionary advantage to cells growing in media containing low concentrations of essential amino acids.

The bacterial cell is, then, enclosed within a membrane displaying selective permeability and possessing mechanisms promoting the transport of substances otherwise unable to diffuse freely through that membrane. Membranes are common to all cells and transport processes have aroused the curiosity of biologists since cells were first recognized. Much work on permeability during the last 10 to 15 years has been carried out with erythrocytes or mitochondria but studies with bacterial cells have contributed significantly to our general knowledge of transport processes.

The membrane

Let us first look at the membrane. Laico, Ruoslahti, Papermaster & Dreyer (1970) tell us that 'cellular membranes serve as active interfaces that govern interactions between cells and their environment and compartmentalize functions within the cell'. We recognize membranes in electron micrographs of cell sections as characteristic tramlines or triple-layer sandwiches. We can see these structures lying beneath the cell wall in sections of bacteria or forming the outermost layer in sections of protoplasts. From whatever source they are isolated, membranes are found to contain proteins and lipids. A wide variety of proteins and lipids is reported in membranes and it appears that there is no composition

characteristic of bacterial membranes as such. In different organisms we find a range of phospholipids – phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine, etc. – containing fatty acids that include straight-chain saturated, straight-chain unsaturated, branched-chain and cyclopropane forms. The proteins include cytochromes and enzymes of the electron-transport system, transport proteins, possibly structural proteins, and the recently discovered ‘mini proteins’ that include cyclic peptides and decapeptides and may make up a high proportion of the total membrane protein fraction*

Fig. 1. Models of membrane structure: (a) 1935 (Danielli & Davson); (b) 1970.

(Bermingham, Deol & Still, 1970; Laico *et al.* 1970). The first model of a membrane, proposed by Danielli & Davson in 1935 (Fig. 1a), was made up of three layers: a middle bilayer of lipid molecules arranged side by side with polar ends outwards, and two outer layers each consisting of protein molecules coating the polar ends of the lipids. The model explained many properties of membranes and could be correlated with the electron micrographs obtained later. It was, of course, too simple to explain the diversity of membranes and has been subjected to much criticism in the 35 years that have elapsed since

* See, however, comment in *Nature New Biology* (1971) **231**, 227.

it was first put forward. Better techniques of study have shown that membrane proteins do not, in general, exist in extended form but are largely in helical conformation, that the lipid core is in a disordered rather than highly orientated state, and that hydrophobic sections of protein penetrate the hydrophobic core so that the structure of the membrane rests on hydrophobic interactions between protein and lipid (Stoeckenius & Engelman, 1969). Chemical, physical and electron-microscope studies provide us with a modification of the Danielli model more along the lines of Fig. 1(b). Whatever the detailed organization at a molecular level, we can see that in order for a substance to pass through a membrane without hindrance or effort it must possess properties which enable it to move through polar and non-polar regions; such substances seem rare. In our early work on amino acid transport in Gram-positive bacteria we found that lysine could enter *Streptococcus faecalis* by diffusion and become concentrated inside the cell by ion distribution (Najjar & Gale, 1950), but the uptake of lysine by other bacteria proves to be an energy-requiring process or to involve more than one mechanism (Gale & Folkes, 1967). In the main we find that nutrients, ions and metabolites can only cross the membrane under quite specific conditions involving the provision and utilization of energy. It was early proposed that these conditions involved some form of chemical modification of the transport substrate, probably combination with a carrier in the membrane, such that the modified form could move through the hydrophobic zone.

Let us now strip the membrane down to its simplest terms and, as MS would certainly have us do, examine the experimental evidence that implicates its various components in transport processes, especially those involving amino acids.

Protein components. Monod and his colleagues were the first to show involvement of specific proteins in the transport of sugars and amino acids across bacterial membranes (Cohen & Monod, 1957). Monod found that certain mutants of *Escherichia coli*, lacking the ability to utilize lactose, possessed β -galactosidase but were essentially impermeable to galactosides. He and his colleagues then showed that the ability to develop a galactoside transport system (a) was under genetic control, (b) involved protein synthesis, (c) was specifically induced by galactosides and (d) that the ability once induced was specific for galactoside transport. Since the transport process thus involved a specific protein with a specific activity, Monod likened this protein to an enzyme and coined the word 'permease' to describe such transport-mediating proteins. Inevitably the word has been misused and taken out of context and, as happens rather frequently, the importance of the discovery has been fogged by dispute over nomenclature; thus Mitchell (1970) complains that 'the permease nomenclature introduced by the Paris school has tended to confuse rather than to clarify the biochemical interpretation of transport phenomena', and instead introduces a series of descriptive terms ranging from ferry-boat to porters and conductors that suggest he has joined another sort of Transport Union altogether. During the last five years the search for methods which would enable a more direct examination of transport-mediating proteins has begun to yield results. Kennedy and his co-workers (Fox & Kennedy, 1965; Fox, Carter & Kennedy, 1967; Carter, Fox & Kennedy, 1968) devised an ingenious technique whereby a β -galactoside-binding protein from *E. coli* could be labelled selectively by *N*-ethyl maleimide, extracted from the membrane fraction and purified. It had a molecular weight of 31,000, each molecule had one binding-site specific for galactosides and it was coded in the y , or 'permease', gene. Further success has been achieved by submitting *E. coli* to osmotic shock (Neu & Heppel, 1965; Nossal & Heppel, 1966) which results in the release of certain proteins from the cell and, at the same time, partial loss of ability to transport certain sugars and amino acids. Examination of the proteins released by the

shock procedure shows that some of them will bind specific sugars or amino acids. Thus Anraku (1968*a*) purified and eventually crystallized one protein which specifically bound leucine and another which specifically bound galactose. In a similar fashion, proteins which bind arginine (Wilson & Holden, 1969), sulphate ions (Pardee, 1966, 1968) or phosphate (Medveczky & Rosenberg, 1970) have been isolated and there is reason to believe that a range of such proteins exists, each having a binding site specific for an amino acid, a sugar or an ion. Those so far obtained in a purified form have molecular weights of 30,000 to 35,000. Shock treatment might remove proteins from the surface or from inside the cell but Nakane, Nicholls & Oxender (1968) have produced immunological evidence to show that the leucine-binding protein lies in the surface of the cell. In some cases the impaired transport systems of shocked cells can be restored to normal by addition of concentrated 'shock fluid' but the more detailed investigations of Anraku (1968*b*) and Wilson & Holden (1969) show that the addition of pure binding-protein or highly purified fractions thereof do not give complete restoration, other fractions from the released material also being necessary. It is probable that the binding proteins constitute a part of the transport process for the sugars or amino acids that they bind but they cannot constitute the whole system nor has their relationship to carriers yet been elucidated. Boos (1969) has reported transport-deficient mutants in which the transport system is damaged by alteration or loss of components other than binding proteins.

Lipid components. In the first place the lipids provide the essential hydrophobic character of the membrane, and we have to ask whether they always act in a purely physical sense or whether any of them can play a metabolic role by reacting with transport substrates to form lipophilic derivatives. Lipoamino acids might be carrier forms of amino acids, and the discovery by Macfarlane (1962) of amino acid esters of phosphatidylglycerol looked promising: however, any transport-mediating role of these substances disappeared when we found that lysyl-phosphatidylglycerol, labelled with ^{14}C in the lysine, of *Staphylococcus aureus* was not diluted during transport of unlabelled lysine across the membrane (Gale & Folkes, 1965). A general biological finding of potential importance is that organisms living at low temperatures have a higher proportion of unsaturated fatty acids in their lipids than organisms living at higher temperatures, and in some cases it has been possible to show that transfer of micro-organisms from high to low temperatures is accompanied by an increase in the degree of unsaturation of their fatty acids (Marr & Ingraham, 1962; Kates & Baxter, 1962). Recently my colleague Mr Russell (1971) has shown in the example of the psychrophile *Micrococcus cryophilus*, in which 95 % of the fatty acids are unsaturated anyway, that a drop in growth temperature from 20° to 0° is accompanied by a fourfold increase in the ratio of C 16 to C 18 monoenoic unsaturated fatty acids. Chapman & Wallach (1968) suggest that changes of this nature are necessary in order to maintain the fluidity of the hydrophobic core of the membrane at low temperatures. Changes in fluidity would affect transport mechanism and it has been suggested (Farrell & Rose, 1967) that limiting fluidity could explain why mesophilic organisms cannot grow below about 10° while psychrophiles continue to transport and grow at much lower temperatures. We have found an effect of lipids on aspartate and glutamate transport in *S. aureus* which can be shown in a number of ways: decrease in the lipid content of the cells by pre-incubation in the absence of a carbon source (Gale & Folkes, 1967), treatment with heroin (Gale, 1970) or shock treatment (Gale & Llewellyn, 1970) is accompanied by decreased aspartate uptake, and the uptake can be restored in each case by addition of lipids extracted from the cells. Hydrolysis and fractionation of the lipid shows that the restoration activity resides in the fatty acid fraction which can be replaced by unsaturated fatty acids (Gale & Folkes, 1967;

Gale & Llewellyn, 1970). Stimulation of aspartate uptake can be obtained in lipid-depleted cells with a wide range of unsaturated fatty acids (16:1 to 22:6), the effect varying with chain length, degree of unsaturation and position of the double bonds in the chain. We have sought for a property of unsaturated fatty acids which could be correlated with their effect on aspartate accumulation. There is no clear correlation with melting point or ability to penetrate monolayers of staphylococcal lipid. However, we have found that small amounts of unsaturated fatty acid prevent the expansion that occurs in such lipid films when the temperature is raised and there is a direct correlation between the amount of fatty acid that will halt thermal expansion of the film and the amount that will produce a given stimulation of aspartate accumulation in cells containing a similar amount of lipid (Gale & Llewellyn, 1971). It is difficult to interpret the significance of this at present but it would seem that the effect on aspartate accumulation can be associated with a physical change in membrane structure. A more specific role for unsaturated fatty acid is suggested by the work of Fox (1969) and Wilson & Fox (1971), who find that such acids are essential for induction of lactose transport but not for the associated synthesis of galactoside acetylase or β -galactosidase in *E. coli*. Fox suggests that the unsaturated fatty acids may bind the transport proteins to the membrane or be involved in the formation of a transport site on the membrane. Wilson & Fox (1971) find that the temperature characteristics of the transport process are determined primarily by the properties of the lipid phase of the membrane. It would seem that we can add unsaturated fatty acids as components of at least some bacterial transport systems, although we must admit that so far we cannot give any real description of what the fatty acids are doing.

Concentration gradients. The next question is: What drives an amino acid into the bacterial cell against a concentration gradient? At least two mechanisms can be put forward: (1) the amino acid as a carrier complex moves up a concentration gradient at the expense of something else moving down a concentration gradient, the energy required for the former process being obtained by dissipation of the potential energy of the latter (facilitated transport in which energy is required to set up the counter-gradient in the first place); (2) the transport process is mediated directly by an energy-rich component built up by the metabolism of the cell (active transport). Studies during the last 5 to 10 years with bacteria, yeasts, tumour cells and isolated mitochondria have yielded considerable information about the first type of mechanism, and its elucidation has been greatly assisted by the use of the group of antibiotics now known, after Pressman, Harris, Jagger & Johnson (1967), as ionophores. If we take our specific example of acidic amino uptake by *Staphylococcus aureus*, it was shown by Davies, Folkes, Gale & Bigger (1953) that the uptake of glutamate was accompanied by uptake of K^+ . This has now been confirmed and extended to aspartate uptake. Biological membranes in general are impermeable to K^+ but it has been found with mitochondria (Moore & Pressman, 1964) and *Streptococcus faecalis* (Harold & Baarda, 1967) that the presence of valinomycin renders membranes permeable to K^+ or Rb^+ but not Na^+ or Li^+ . The action of valinomycin is a specific one on K^+ transport, and Harold & Baarda (1968) have shown that the antibacterial effect of such ionophores can be explained in terms of depletion of K^+ in the cells with consequent cessation of protein synthesis. Since valinomycin renders membranes permeable to K^+ , it results in equilibration of any K^+ gradient previously existing across the membrane and will consequently prevent translocation of any substance whose movement is dependent on, and coupled to, dissipation of such a gradient. Thus Harold & Baarda (1967) found that valinomycin stopped the uptake of phosphate and glutamate by *S. faecalis*; we have confirmed these findings and extended them to the uptake of aspartate and alanine but

not lysine. The inhibitory effect of valinomycin on growth and these transport processes in *S. faecalis* can be antagonized by high concentrations of K^+ as shown in Fig. 2.

Fig. 2 also shows, however, that a different situation arises with *Staphylococcus aureus*. Valinomycin has little inhibitory effect on the uptake of aspartate or glutamate unless a high concentration of K^+ is added to the incubation medium. Valinomycin (10^{-6} M)

Fig. 2. Effect of potassium ion concentration on the inhibition of aspartate transport by valinomycin in *Streptococcus faecalis* and *Staphylococcus aureus*.

becomes inhibitory when the concentration of K^+ in the medium rises above that in the cells (approximately 10 mM); inhibition approaches 90 % when the concentration of added K^+ is 300 mM. This suggests that the uptake of aspartate is coupled to K^+ translocation when there is a gradient of K^+ concentration from outside to inside the cell. However, in the absence of antibiotic the uptake of aspartate is not dependent upon, nor significantly affected by, the presence of K^+ ; it follows that aspartate transport is mediated by some factor in addition to K^+ . Fig. 3 shows that the rate of aspartate uptake, and the concentration gradient eventually attained, are related in a linear fashion to the external proton concentration over the range pH 5.5 to 8.5. Working with cells at pH 5.5 in the absence of glucose we have been able to show a proton uptake amounting to 0.65 H^+ equivalents/molecule of aspartate or 0.9 H^+ equivalents/molecule of glutamate taken up by the cells. If K^+ is added to the external medium, then the aspartate uptake becomes sensitive to valinomycin and the degree of inhibition increases as the external proton concentration decreases. According to Mitchell (1970), 2,4-dinitrophenol acts as a proton conductor in membranes and equilibrates proton gradients in a way similar to that of valinomycin in destroying K^+ gradients; dinitrophenol abolishes aspartate uptake in *S. aureus*. The sensitivity to dinitrophenol is decreased by the presence of K^+ while the sensitivity to valinomycin is decreased by increasing the H^+ concentration. In *S. aureus* aspartate uptake

is coupled to proton translocation but at low external H^+ concentrations and high external K^+ concentrations the process becomes sensitive to valinomycin. Whether we can regard H^+ and K^+ as co-substrates for the aspartate carrier or whether a more complex situation arises – such as a K^+/H^+ antiporter coupled to a H^+ /aspartate symporter under appropriate conditions – is now being investigated.

Fig. 3. Effect of pH on the uptake of aspartate and its inhibition by valinomycin in *Staphylococcus aureus*. Curve 1 = concentration gradient achieved after 15 min. at 15°. Curve 2 = concentration gradient achieved after 60 min. at 15°. Curve 3 = % inhibition of uptake by 1 μ M valinomycin (incubation medium contains 100 mM KCl). Ordinate: empirical value of concentration gradient across cell membrane; $P/S = 1$ corresponds to an internal aspartate concentration approximately 1000 times that in the external medium (see Gale & Folkes, 1967).

Ionophore action. Let us now look more closely at valinomycin and the nature of its action as K^+ conductor. Valinomycin is a cyclic depsipeptide of molecular weight about 1100 containing three residues each of L-valine, D-valine, L-lactic acid and D- α -hydroxyisovaleric acid. Pressman (1965) and Shemyakin *et al.* (1969) have shown a high specificity in its structure; open chain equivalents or larger or smaller rings with the same sequence are inactive; the sequence of D- and L-residues and the presence of hydroxyvaleric acid are essential, although some variation in the nature of the hydrophobic amino acid is permitted. The molecule can be constructed in a way that produces a disc with hydrophobic residues on one side and hydrophilic residues on the other; as such it would tend to align itself in the interface between lipid and aqueous phases. Ivanov *et al.* (1969), Shemyakin *et al.* (1969) and Ohnishi & Urry (1970) have shown that the ring undergoes buckling to form a 'bracelet' which then accommodates a K^+ atom to form a lipid-soluble complex. The carbonyl oxygens of the interior of the bracelet replace the oxygens of water so that the hydration shell of the K ion is replaced by an organic shell with a lipophilic exterior (Fig. 4). When K^+ enters the valinomycin molecule to form the co-ordinate complex the molecule

Fig. 4. Structure of valinomycin and its potassium complex (Pinkerton, Steinrauf & Dawkins, 1969).

as a whole undergoes a change in conformation so that it becomes more lipid-soluble and will sink from the interface into the bulk of a lipid medium. In other words, the design of valinomycin is such that it will sit in the surface of a membrane in a conformation that allows it to take up a K ion and the K⁺-complex then formed will sink into the core of the membrane and can shuttle across the hydrophobic barrier. The K⁺-valinomycin complex is unstable in the presence of water and will dissociate when it comes into contact with the aqueous interior of the cell; in the presence of a K⁺ gradient, the shuttle system will carry K⁺ down the gradient until equilibration is achieved. Valinomycin is therefore a carrier in the classical sense and facilitates diffusion of K⁺ across natural and artificial membranes.

Monensin and nigericin are further examples of antibiotics that act as ion conductors in membranes. They differ from valinomycin and other ionophores in that they are acidic and not cyclic molecules. Pinkerton & Steinrauf (1970) have shown that, in the deprotonated form, these antibiotics possess an open circle conformation, stabilized by hydrogen bonds, which forms lipophilic co-ordination complexes with cations. These are examples of structures which act as cation conductors only after deprotonation so that, for example, H⁺ and K⁺ translocations are tightly coupled and monensin can be regarded as a model for Mitchell's symporter or antiporter. It is conceivable that the aspartate carrier in *Staphylococcus aureus*, where protons are essential, is of a related nature.

Pressman *et al.* (1967) first pointed out that ionophores can be regarded as carriers taking part in a facilitated diffusion and, further, that if the formation of the cation-ionophore complex required energy then such an ionophore could be regarded as a model for active transport. This may be the case with alamethicin, a large and flexible cyclic peptide (Payne, Jakes & Hartley, 1970) which acts as a cation conductor whose activity is modified by the potential applied to the membrane and by the presence of substances such as peptides, amines and certain energy-rich substances. Six or more alamethicin molecules are involved in the transport of one cation and it may be that the cyclic peptide structures form a pore or channel through which cations flow, while various chemical, electrical and energetic factors affect the assembly or disassembly of the aggregate forming the pore (Mueller & Rudin, 1968). It is of considerable interest that Hauser, Finer & Chapman (1970) have shown that alamethicin induces changes in the lipid organization of bilayer systems.

A scheme for facilitated transport. Now we can engage in some speculative extrapolation: if an ionophore such as valinomycin or monensin possessed an amino acid-binding site which was unmasked as a result of conformation change on complex formation with K⁺ or H⁺, then we should have a molecule which would act as an amino acid conductor moving down, and coupled to, a cation gradient. We can ask whether antibiotic ionophores are produced as a result of excess production of natural conductors in the membranes of the cells that form them, or whether they are analogues of natural carriers present in all membranes. Laico *et al.* (1970) and Bermingham, Deol & Still (1970) have recently reported the presence of small cyclic peptides and depsipeptides in erythrocyte and bacterial membranes, while Blondin, DeCastro & Senior (1971) have isolated a neutral, cyclic dodecapeptide by extraction of beef heart mitochondria with organic solvents and shown that the purified peptide is an ionophore that facilitates the transport of sodium and potassium across the mitochondrial inner membrane. It seems reasonable therefore to include cyclic ionophores, probably peptide in nature, as components of our transport mechanism. If we draw evidence from the various systems I have discussed, we can picture a scheme for the facilitated transport of an amino acid, such as aspartate, across a membrane. The scheme

includes (1) a specific binding-protein which captures the amino acid at the outer surface of the membrane and holds it in a position where it can react with (2) a carrier with the properties of an ionophore possessing binding-sites for the amino acid and a metal-ion or proton (the amino acid binding-site possibly being unmasked as a result of a conformational change following complex formation with a cation); the movement of the cation-carrier-amino acid complex will then be dependent on (3) a cation gradient of the appropriate sense, and (4) involve an organization of the hydrophobic core of the membrane dependent, in a manner not understood, on the presence of unsaturated fatty acids. Any scheme based on these components is probably incomplete and, unless biochemical history is bunk, certainly not too complex. Investigations of the behaviour of various ionophores in artificial membrane systems suggest that the mechanism of conduction across the hydrophobic core may differ with different ionophores (see, for example, Haynes, Kowalsky & Pressman, 1969; Keller-Scherlein & Simon, 1969; Hladky & Haydon, 1970). Our scheme is based, albeit somewhat loosely, on experimental evidence and, as such, would receive the approval of MS.

It may seem curious to give a memorial lecture on a topic specifically abjured by the person whose memory we honour but one of MS's outstanding traits was her willingness to admit that she was wrong and to reconsider her judgement of people and things. Scientific enthusiasm involves setting up concepts and knocking them down and our certain heritage from MS is enthusiasm for our subject. I know that she would be delighted with the situation that has developed around the problems of permeability – not only for the beauty of the biochemistry that has been revealed but for the diverse and magnificent challenge it presents for future work. Today I am sure her comment would be, 'Now perhaps we *can* talk about permeability.'

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