III. FUNDAMENTAL BIOPHYSICAL ISSUES

The concepts of the structure and chemistry of the brain cell microenvironment outlined in the previous chapter form a basis for discussing both biophysical and physiological questions. The next three chapters are devoted to the biophysics of the brain cell microenvironment. In this chapter, Gardner-Medwin presents an essay on the fundamental issues, which incorporates ideas that he has developed over several years. The essay was largely formulated in the aftermath of the Work Session and provides a conceptual framework for the two subsequent chapters, which deal with the experimental facts on the migration and homeostasis of ions and macromolecules in the brain and the importance of water relations.

Membrane Transport and Solute Migration Affecting the Brain Cell Microenvironment*: A.R. Gardner-Medwin

Fluctuations of neuronal activity lead to changes of the concentrations of certain substances in the EC space, notably K^+, Ca^{2+}, and transmitter chemicals. The dynamics of the fluctuations may involve active and passive transport across the membranes of cells, diffusion in the EC space, transport across the blood-brain barrier, water movement between different compartments, and the effect of voltage gradients within the tissue. A full understanding of the interplay of these different factors is probably beyond our present grasp. Advances in experimental technique, particularly the introduction of ion-selective microelectrodes, however, now permit a more quantitative and theoretical analysis.

Two views have emerged about the possible importance of understanding the dynamics of the brain cell microenvironment (Somjen, 1979a). The first is that the stability of brain function requires the maintenance of the neural environment within strict limits and the minimization of the effects induced by active neurons upon their neighbors. The second view is that the interactions between neurons mediated by changes in the EC space constitute important aspects of neural function. Both views may of course be correct in applying to different structures within the brain. Even within a single structure, for example the glial envelope around synaptic glomeruli (Peters et al., 1976), it is possible that the morphology could be such as to maximize the interactions via the EC space within the glomerulus while at the same time preventing this region from influencing outside tissue or being influenced by it (see also Ellisman, Chapter II).

Parts 1 through 9 of the following section discuss some of the ways in which ion flux and redistribution can affect the composition of the fluid within the brain cell microenvironment. The purpose is to outline some of the more important quantitative relationships between different processes. There are numerous types of experiments that bear on these problems and many conflicting interpretations. Although several of the issues are of general importance, emphasis is placed on the study of potassium because it was clear at the meeting that this is a topic of special significance.

*This section is an expanded discussion of issues raised at the Work Session. It contains hitherto unpublished material which is central to the topic of the Work Session, and it was considered appropriate to invite Dr. Gardner-Medwin to contribute the section under his own name. The suggestions of Dr. Julian Jack, who also reviewed this section, are gratefully acknowledged.

1. Space-independent and Space-dependent Processes Affecting Extracellular Solute Concentrations

We are concerned here with the different types of processes that come into play when there is a disturbance of the brain cell microenvironment from its steady resting state. It is necessary to make a fundamental distinction between processes (and the equations that govern them) that are *space-independent* and those that are *space-dependent*.

If a solute is added to the baseline composition of the EC space, the excess concentration will not necessarily remain constant. It may change, for example, as a result of uptake into cells or transfer across the capillary walls. These are both examples of *space-independent* processes: they depend on the amount, and possibly on the previous time course, of the concentration excess; but they will affect the EC concentration in the same fashion whether the concentration excess is local or is uniform throughout the tissue. Such processes can result in a redistribution of solutes among different compartments within the tissue, or between the brain and the blood, but they are not associated with any flux of solute through the tissue. The three general types of space-

independent processes considered here are indicated diagrammatically in Figure 9A, 1–3. They include the two processes mentioned above and, in addition, changes of the relative volumes of intracellular space and EC space, which directly affect the intracellular and EC solute concentrations.

If the disturbance of solute concentrations is not uniform (within a single tissue compartment), then there are gradients through the tissue and *space-dependent* processes will lead to fluxes of solute through the tissue. An example of this is when solute is released locally into the EC space and the resultant increase in EC concentration falls as the solute diffuses away. In this case the EC concentration is affected by *dispersal* (a flux away from the point of release), in addition to any space-independent processes that may remove solute from EC space. The processes considered here that can result in fluxes through the tissue are diffusion in the EC and intracellular space, bulk flow of solution, and the transfer of ions associated with electric current flow (Figure 9B, 4–7). None of these processes will contribute to the dynamics of the

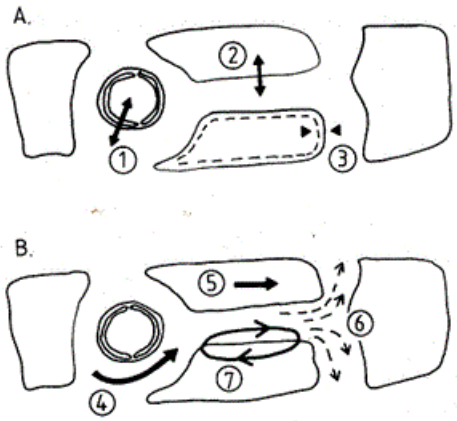

Figure 9. Mechanisms affecting the concentration of extracellular solutes: A. Mechanisms involving different compartments, but independent of gradients between one region of tissue and another. 1, Solute exchange across capillary walls; 2, Solute exchange between intracellular and extracellular compartments; 3, Concentration changes consequent on movements of water across cell membranes. B. Mechanisms dependent on gradients and capable of causing solute flux through the tissue. 4, Extracellular diffusion; 5, Intracellular diffusion; 6, Bulk flow of extracellular solution; 7, Transport of ions associated with current flow. [Gardner-Medwin]

brain cell microenvironment when a disturbance is everywhere uniform; but their effectiveness may be an important factor in determining the size and duration of changes in the brain cell microenvironment caused by a local disturbance.

Quite complicated issues arise if one tries to consider the relative importance of different processes in diminishing the consequences of a disturbance of the brain cell microenvironment. The importance of different processes in general depends on both the duration and the distribution of the disturbance. Thus it has been argued by Gardner-Medwin and his colleagues that the major part ($> 80\%$) of a K^+ flux maintained over large distances through brain tissue is carried through cells (mostly by mechanism 7 in Figure 9B), while over short distances (less than a few hundreds of μm in rat brain, a few tens of μm in frog brain) EC diffusion becomes more important. This conclusion unfortunately does not demonstrate whether dispersal processes of either type are important in practice compared with space-independent processes such as active or passive uptake into cells. Heinemann (see Chapter IV) and others have argued that active transport into cells is important in the clearance of excess K^+ in EC space, and accounts for the undershoot of $[K^+]_o$ which often follows a period of $[K^+]_o$ elevation. Coles and his colleagues (see Chapter VI) have shown that substantial amounts of K^+ can build up within glial cells when there is a release of K^+ into EC space in an invertebrate preparation. When one wishes to compare the relative importance of these various mechanisms, it would be ideal in principle if one could eliminate each mechanism separately and observe the effect on the changes of EC concentrations caused by a particular disturbance. Such elegant experiments are rarely possible however; for example, if one abolishes active transport by metabolic or specific blocking agents, by the time one is sure that the block is complete there may be substantial changes in EC and intracellular ionic composition and of EC space fraction. Despite much debate at the Work Session, the need was still felt for a clearer framework for attacking the problem of the relative importance of different mechanisms. Many of the general issues are discussed in the following sections.

2. Exchange Across the Capillary Walls

Exchange between extracellular fluid and the blood constitutes the means by which the normal baseline composition of the brain fluids

is maintained constant over long periods of time. The nature of this exchange, both at the cerebral capillaries and also via the cerebrospinal fluid secreted at the choroid plexus, has been the subject of much detailed study (see, e.g., Davson, 1970). The critical question in the context of this Work Session, however, is whether the transport and regulatory mechanisms of the blood-brain barrier can react to a disturbance of the EC composition fast enough to be relevant to transient disturbances within brain tissue.

Probably no region of neural tissue in the mammalian brain is more than 25 to 50 μm from a capillary (see, e.g., Purves, 1972). This means that, even allowing for the tortuosity and restricted space of extracellular fluid, any disturbance of the EC concentration of small molecules or ions will spread to within range of the capillaries within at most a few seconds. This is in practice never likely to be the rate-limiting step in exchange with the blood.

The rate of exchange with plasma is affected by two principal factors: (1) the permeability of the capillary walls, (2) the blood flow rate. If we consider substances that pass readily across membranes (e.g., H_2O, O_2, Xe), the blood flow is rate limiting. This forms the basis, indeed, for some of the techniques for measuring cerebral blood flow (Purves, 1972). With such substances the venous blood is always fully equilibrated with the EC space, and the composition of the tissue will approach equilibrium with arterial blood with an exponential time constant approximately equal to the reciprocal of the cerebral blood flow rate (assuming a 1:1 partition coefficient for the substance between brain and blood). Typical blood flow rates in mammals are 0.5 to 1.0 ml blood per ml tissue per min, giving a time constant of 60 to 120 sec. Apart from O_2 and CO_2, which probably have a higher solubility in blood than in brain, it is unlikely that any substances exchange across the blood-brain barrier with a substantially shorter time constant than this, and it is probably much longer for most of the substances we are interested in (see, e.g., Oldendorf, 1975).

Ions in general do not pass easily across biological membranes, and indeed the time course for equilibrium with brain tissue of radioactive tracer ions in the blood is normally extremely slow (of the order of hours; see, e.g., Fenstermacher, 1975). In the cases of Na^+ and Cl^- (which are largely EC ions), this time course represents fairly directly that of exchange between the blood and the EC fluid. For potassium, which is largely an intracellular ion, the time course of exchange is extremely slow (half-time about 24 hours) but nevertheless corresponds to a capillary permeability that is 10 times higher than for Na^+ and Cl^- (Davson and Welch, 1971) and would result in diffusional equilibration between blood and EC space *alone* in about 15 to 30 min (i.e., if exchange with intracellular K^+ did not occur). In practice, changes of $[K^+]_o$ are never likely to occur for this length of time without substantial equilibration with intracellular space (see below). Thus even for potassium, the time course with which capillary diffusional exchange can affect the EC concentration must be at least many tens of minutes.

The exchange of ions between EC fluid and blood is not, in fact, a purely passive diffusional exchange, since it maintains the concentrations of many ions in the brain cell microenvironment constant in the face of substantial changes in the plasma (see, e.g., Kuffler and Nicholls, 1976). In the case of K^+ there is evidence that uptake of radioactive K^+ from the blood is sensitive to the K^+ concentration in EC fluid (Bradbury et al., 1972); this may indicate that active transport across the capillary walls is stimulated by abnormal EC K^+ levels. The magnitudes of the changes of uptake (+44% for perfusion of the brain surface with K^+-free fluid), though substantial, are not sufficient to alter the order of magnitude of the time course with which one can infer that the composition of EC space would be affected.

In summary, it seems unlikely that the blood-brain barrier plays a major part in the dynamics of the brain cell microenvironment except where equilibration of water, O_2 and CO_2, or other highly permeant substances are concerned, or where effects extending over many tens of minutes are involved.

3. Exchange of Ions Between Intracellular and Extracellular Compartments

The exchange of ions across cell membranes is the cause of many of the transient disturbances of the brain cell microenvironment. For example, neural activity may be associated with influxes of Na^+, Ca^{2+}, Mg^{2+}, and Cl^- and with effluxes of K^+ and synaptic transmitters. Eventually the normal resting state of the cells causing the disturbance is restored; for the inorganic ions this involves simply a reversal of the solute flux across the membranes, usually by active transport. Full recovery probably requires a few minutes, as judged by the time course

of the electrical and metabolic after-effects of stimulation in nerve (see Cohen and De Weer (1977) for review) and in brain slices (McIlwain and Bachelard, 1971).

In this section we are concerned with membrane exchange processes that can act to reduce the disturbance of the brain cell microenvironment before the normal cellular equilibrium is restored. During this time many additional cells (including both neurons and glia) will be exposed to the altered EC fluid and may act to buffer changes in its composition through temporary exchange across their own membranes.

The total membrane area available for exchange between EC and intracellular compartments is large in nervous tissue because the cellular components are mostly very fine cylindrical or sheetlike processes. Horstmann and Meves (1959) calculated from electron micrographs a total area of plasma membrane per unit volume of tissue of about 5 μm^{-1} (i.e., 5 μm^2 per μm^3 of tissue), corresponding roughly to an average diameter of cell processes of 1.2 μm. The intercellular clefts in this study were probably unphysiologically narrow as a result of fixation (Van Harreveld, 1972), but this distortion should not have affected appreciably the measurements of total membrane area. If we take current estimates for the EC space fraction in brain, as discussed by Van Harreveld (1972), we can conclude that the ratio of membrane area to *extracellular* volume is of the order of 20 μm^{-1}. This figure will be used in the ensuing discussion of individual exchange mechanisms where it enters into calculations.

Any change of cell membrane potentials implies a *capacitative shift* of ions between EC and intracellular space. Assuming a capacitance of 1 $\mu F \cdot cm^{-2}$ and a ratio of membrane area to EC volume of 20 μm^{-1}, this shift accounts for a change of EC concentration of 0.2 mM of univalent ions for each 100-mV change of membrane potential. Such shifts are a major component of the Na and K fluxes associated with action potentials. They are too small, however, to contribute significantly to the buffering of ion changes in the EC space. Addition of K^+ to the EC space will cause cells to be depolarized; but even if (as in glial cells) the depolarization amounts to the full change of the Nernst equilibrium potential for K^+, the capacitative shift of K^+ from the EC space will only serve to diminish the rise of $[K^+]_o$ by 2%.

An ion flux between EC and intracellular space represents a current flowing across the membrane. If such a flux is to exceed the amount attributable to the capacitative shift, then the current in one

direction must be balanced by current in the opposite direction across the membranes. This balancing current may come about either through flux of a second ion species (further discussed in this section) or it may occur through flux of the same ion species in the opposite direction across a different region of the cell membrane. The second of these possibilities (Figure 9B, 7) is a space-dependent process requiring some sort of gradient through the tissue; its contribution to K^+ dispersal is known as the "spatial buffer" mechanism (and is discussed in Part 9). Wherever space-independent mechanisms cause significant ion flux between EC and intracellular compartments, there must also be flux of at least two ion species.

There are several pairs of ion species that can reequilibrate when there is a disturbance of the brain cell microenvironment. Only those involving K^+ will be considered here, since these are probably the most important for the buffering of the EC space. If the EC concentration of K^+ is raised, several changes will occur in cells whose cytoplasmic contents are initially normal. The principal immediate effects will be: (1) passive K^+ influx leading to depolarization; (2) stimulation of active Na^+/K^+ exchange pumping by the increased EC binding of K^+ to pumping sites, leading to Na^+ efflux and K^+ influx; (3) changes of passive ion fluxes consequent on depolarization: a decrease of Na^+ influx and an increase of Cl^-, HCO_3^- influx.

The best understood mechanism for paired ion movement is probably the passive uptake of KCl (a combination of (1) and (3) above) during the attainment of a new Donnan equilibrium (Boyle and Conway, 1941). The Cl^- influx due to a decrease of the resting potential is eventually counteracted by the rise of $[Cl^-]_o$. Boyle and Conway showed in frog muscle that the increase of KCl concentration in the cytoplasmic water at equilibrium was closely equal to the increase of KCl concentrations in EC fluid, when $[Na]_o$ was kept constant. If $[K^+]_o$ was increased by substitution for $[Na^+]_o$, the same KCl uptake occurred into the cells, but in this case there was cellular swelling with no change of $[K^+]_i$. The results were consistent with an average charge of (−1.0) on the impermeant molecules of the cytoplasm, as inferred from analysis of the muscles. Similar results have been obtained for K/Na substitution in crayfish axons (Wallin, 1967). There are too many uncertainties to allow these conclusions to be applied directly to brain tissue. Finkelstein pointed out, for example, that Cl^- is not in passive equilibrium across the membranes of some cells. But the observations

suggest that if KCl entry occurs rapidly enough, it could account for substantial buffering of changes in $[K^+]_o$. An initial increase of $[K^+]_o$ would at equilibrium be diminished by about 75% if we imagined that the cells of neural tissue behaved like the sartorius muscles of the frog or like crayfish axons.

The time course of approach to a new Donnan equilibrium is determined by the membrane slope conductance for both the ions concerned. Thus if we assume even a fairly high specific membrane resistivity of 5 $k\Omega \cdot cm^2$ for all the membranes bordering the EC space (taken at 20 μm^2 per μm^3 of EC volume) and we assume that the membrane conductance is divided solely between K^+ and Cl^- in the ratio r : 1 or 1 : r, then it can be shown* that the time constant for exponential approach to equilibrium when there are small displacements of $[K^+]_o$ is approximately 1 sec for r = 1, 3 sec for r = 10, and 24 sec for r = 100. The Cl^- conductance of neurons of the CNS is uncertain and varies, for example, with pH and with transmitter action. But the K/Cl conductance ratio probably falls within the stated range (r=25 for squid axons: Brinley and Mullins (1965); r = 4 for cat motoneurons: Eccles (1957)). Coombs and colleagues (1955) measured the time constant for recovery from iontophoretic injections of Cl^- into motoneurons and found it to be about 30 sec. If motoneurons were typical of all the cells bordering the EC space, the time constant for removal of KCl from the relatively small volume of the EC space by equilibration with cytoplasm should be about 5 times smaller, or 6 seconds. Much of the membrane bordering EC space, however, belongs to glial cells, which may behave differently from neurons. The contribution of glia to the uptake of KCl from EC space is uncertain. Their Cl^-/K^+ conductance ratio is believed to be low, but, on the other hand, their total membrane conductance is probably high (see Orkand, 1977). There is some evidence that glia may account for the major component of KCl uptake into neural tissue (see the discussion by House, Chapter V). It is certainly feasible that neurons and glia together could account for a KCl uptake from the EC space that is both substantial and fairly fast, reaching equilibrium within a few seconds.

It is uncertain whether HCO_3^- entry into cells is likely to assist K^+ uptake to any significant extent. Bicarbonate ions entering into cells would probably mostly be removed by intracellular acid-base buffering, resulting in a change in the net charge on the proteins and some increase of intracellular pH. Net HCO_3^- entry would ultimately be limited probably by stimulation of the membrane transport processes by which intracellular pH is maintained. Since too little is known about these various factors in the CNS or about HCO_3^- permeabilities, speculation at present about the significance of HCO_3^- movements, or of the closely related H^+ or OH^- movements, is not likely to be profitable.

*A.R. Gardner-Medwin, unpublished calculations.

The factors affecting Na^+ flux are reasonably well understood, although they are rendered complicated by the major role of *active transport* in Na^+ balance. In cells with raised intracellular Na concentration (e.g., active neurons), active transport will be stimulated so as to pump Na^+ out and K^+ in. The pumping rate should be approximately proportional to the $[Na]_i$ for any given $[K^+]_o$ (Glynn and Karlish, 1975). Active transport stimulated in this way may account for the lowering of $[K^+]_o$ levels below baseline, which is sometimes observed after stimulation of neural tissue (see the discussion by Heinemann, Chapter IV). It cannot be regarded strictly as a process acting to buffer the changes in EC composition, because it is part of the process reversing the initial change that has caused a disturbance of the brain cell microenvironment.

Active transport may also be stimulated by a rise in $[K^+]_o$. This will occur in cells that have not themselves been active and that are affected only by the disturbance of the composition of the EC space. The effect of $[K^+]_o$ on pumping may be more marked, or effective up to higher K^+ concentrations, in glia than in neurons (e.g., Franck et al., 1978). The stimulation of active transport will aid the clearance of K^+ from the EC space until either the $[K^+]_o$ levels are brought back to baseline or the cells are sufficiently depleted of Na^+ that the EC activation of pumping is counteracted by the lowered $[Na^+]_i$. In this case, since stimulation of the pump is due to the rise of $[K^+]_o$, it should not be expected to contribute to undershoots of $[K^+]_o$ below baseline. The total uptake of K^+ by this means could account for an increase of $[K^+]_i$ anywhere between zero and several times the change of $[K^+]_o$: the latter being the case, for example, if the pumping rate were proportional to both $[Na^+]_i$ and $[K^+]_o$, and the normal $[Na^+]_i$ concentration were much higher than the $[K^+]_o$.

Na/K exchange across membranes may also result from changes in the *passive fluxes* when there is an elevation of $[K^+]_o$, even without stimulation of active transport. The depolarization induced by an increase in $[K^+]_o$ reduces the inward driving force for Na^+ and (unless there is a substantial increase of Na permeability) thereby reduces the

passive Na^+ influx. The active Na^+ efflux, which previously equalled the influx for a cell in resting condition, now exceeds the influx and there is net loss of Na^+. This efflux of Na^+ will terminate when the pumping rate is reduced by $[Na^+]_i$ depletion. In cells with a high ratio of $[Na^+]_i$ to $[K^+]_o$, the fall of $[Na^+]_i$ due to this mechanism would be substantially larger than the change in $[K^+]_o$. Thus, a significant buffering of $[K^+]_o$ could occur through passive Na/K exchange across membranes with, in fact, a diminution of active Na^+ efflux.

The conclusion of the last paragraph must be qualified because of uncertainty about the dependence of Na permeability on membrane potential in brain cells. Increases of Na permeability may be induced by K^+ depolarization in neurons (either through changes in the resting permeability or through the transient increases associated with a higher rate of action potential firing). These increases may be enough to cause a net Na^+ influx and K^+ efflux despite the reduced inward driving force for Na^+. This positive feedback mechanism (from the point of view of changes in $[K^+]_o$) is the opposite of a clearance process tending to buffer the $[K^+]_o$. If it comes to outweigh the clearance processes, it may lead to a high regenerative increase of $[K^+]_o$, as has been proposed for the mechanism of spreading cortical depression (Grafstein, 1956; Chapter VII of this *Bulletin*). The possibility that the $[K^+]_o$ would be intrinsically unstable in neural tissue without the existence of the various mechanisms for uptake and dispersal discussed here is a major reason for the great interest in this field.

Data on the effects of $[K^+]_o$ on $[Na^+]_i$ suggest that the balance of the mechanisms discussed in the last three paragraphs is to produce a net loss of cellular Na^+ when $[K^+]_o$ is elevated. In crayfish axons a small Na^+ loss was observed, but enough only to account for a rather insignificant K^+ uptake compared with that occurring in association with chloride (Wallin, 1967). In brain slices, increases of $[K^+]_o$ up to 20 mM have been shown to lead to quite substantial sodium loss, accounting for about half the potassium gain (Lund-Andersen and Hertz, 1970). These are results from experiments with prolonged equilibration.

The time course of Na/K exchange remains rather uncertain; it can be expected to depend on the slope conductance for Na^+ in much the same way as is discussed above for Cl^-. Calculations made with simple assumptions suggest that, for any given cell type, the time constant with which it would equilibrate with the EC space would be smaller than the time constant for it to recover from internal sodium loading by a factor of about 0.2 on account of the restricted EC space, and a further 0.2 if $[K^+]_o = 3$ mM, $[Na^+]_i = 50$ mM, $V_m = -75$ mV. Thus any equilibration of EC space by Na/K exchange with neurons could occur about 25 times faster than the recovery of active neurons and would be fast enough to significantly reduce the $[K^+]_o$ levels during recovery. Once again, the likely role of glia in these processes is unclear but may be substantial.

To summarize: Following neural activity we may have several types of ion fluxes between EC and intracellular space, which contribute significantly to the dynamics of the EC concentrations. Active transport eventually pumps Na^+ out of neurons that have been active and restores their intracellular K^+. But probably there are several processes with a faster time course, possibly involving both neurons and glia, that act to take up K^+ temporarily into cells not affected during the initial disturbance. These buffering processes (probably mainly KCl uptake and Na/K exchange) can occur as the result of purely passive fluxes, though there may be an additional contribution through stimulation of active transport. Their importance may be that they ensure that the net effect in the tissue of a rise of $[K^+]_o$ is an influx of K^+ into cells rather than an efflux, which would lead to instability through positive feedback. A quite rapid drop of $[K^+]_o$ (taking a few seconds) has been observed after stimulation in situations where dispersal processes probably play little part (e.g., Vern et al., 1977; Cordingley and Somjen, 1978). It seems likely that both passive and active uptake processes may contribute to this fall and that the cells involved are not only those that have themselves been active. Such processes should probably be taken into account in interpreting the data on the effects of exogenous K^+ applied by cortical superfusion (Fisher et al., 1976) or by iontophoresis (Lux and Neher, 1973; Nicholson et al., 1979).

4. Osmotic Effects

Water relations are considered in this *Bulletin* chiefly in Chapter V; therefore, these comments will be confined to the influence of osmotic effects on the concentrations of solutes in the EC space.

The simplest disturbances of the brain cell microenvironment (due, for example to Na/K exchange across active neural membranes) probably involve little change in the total number of osmotically active particles in the tissue. Water may move across cell membranes in consequence of the ion fluxes, but there is likely to be little change in the total tissue volume or in the osmotic pressure of the EC space. Movements of water across cell membranes (for example, during the uptake of isotonic KCl into cells when $[K^+]_o$ is raised) result in changes in the concentrations of the solutes which do not move. The percentage changes are larger in EC than in intracellular space, because EC volume is approximately one-fourth as large as the intracellular volume. Evidence was discussed at the Work Session by Heinemann (Chapter IV) and by Nicholson (Chapter VII) that significant diminution of EC space fraction can be observed after activity and during spreading depression.

Changes in the number of osmotically active particles within cells (e.g., associated with metabolism) or changes in the osmotic/charge ratio of the impermeant anions within cells (e.g., associated with pH changes) can lead to passive shifts of water and of ions. The consequences could be slightly paradoxical, in that, for example, a shrinkage of EC space can result, together with a reduced $[K^+]_o$. This could occur simply through the Donnan redistribution process, which would be initiated by a transfer of water from the EC to the intracellular compartments. A true Donnan equilibrium for K^+ and Cl^- can be expressed by equality of the concentration products: $[K^+]_o[Cl^-]_o = [K^+]_i[Cl^-]_i$. The principal effect of the water movement is on the EC concentrations, increasing both terms in the product $[K^+]_o\ [Cl^-]_o$. KCl will enter the cells in consequence until the EC concentration product falls roughly back to normal (more strictly, to equal the intracellular products; but this has been altered much less than the EC product). The loss of KCl from the EC space has a much larger proportionate effect on $[K^+]_o$ than on $[Cl^-]_o$, because the absolute values are so different. Thus the final state is one with $[Cl^-]_o$ elevated, $[K^+]_o$ reduced below normal, and with lowered EC space fraction. Each parameter is altered by approximately the same percentage, either upwards or downwards. This effect is of interest because it could possibly contribute to the undershoots of $[K^+]_o$ sometimes observed after neuronal activity. Thus, although such undershoots are most easily related to active transport of K^+ into neurons (see part 3 of this section and Heinemann, Chapter IV), this may not be the only mechanism.

5. Diffusion of Substances Through the Clefts Between Cells

The physicochemical nature of the EC space and its possible role in influencing the diffusion and binding of substances within the brain cell microenvironment were major topics under discussion at the Work Session (Chapters II and IV). As far as diffusion is concerned, the conclusion was that there is probably little discrimination between different small molecules or ions; this is consistent with the idea that the absolute mobilities of these substances within the clefts are the same as in free aqueous solution. The EC space is a region with complex geometry, however, and this introduces factors that must be carefully handled and that have in the past probably led to some confusion.

The geometry of the EC space affects ion movement in two ways: (1) through the *tortuosity* of the available channels, and (2) through the *restricted space* for movement. The effects of these two factors are quite different and, in principle, independent. Artificial composite media could exist in which each factor operated without the other: media containing infinitely thin baffles and ones with straight pores in the direction of ion movement.

The effect of *tortuosity* on macroscopic diffusion and mobility is equivalent to a nonspecific reduction of diffusion coefficients by a factor λ^2 so that the apparent diffusion coefficient D^* becomes $D^* = D/\lambda^2$, where D is the diffusion coefficient in an unobstructed medium of EC fluid. In simple situations λ is the factor by which the diffusion pathlength is increased (see Appendix of Safford and Bassingthwaite, 1977). Within anisotropic regions of the brain, λ probably varies in different directions. Measurements with various radioactive EC tracers using Fick's Second Law of Diffusion (see below) have given $D^* \simeq D/2.5$ for diffusion from the surface of mammalian brain (Fenstermacher et al., 1974). A tortuosity factor $\lambda^2 = 2.5$ is quite plausible for neural tissue (cf. $\lambda^2 = 2.0$ for close-packed hexagonal prisms), though it would be laborious to predict it accurately from anatomical data. Lack of evidence of a major discrepancy with tortuosity theory supports the idea that molecular diffusion within the clefts is substantially similar to diffusion in water.

The *restricted EC space* (EC space fraction $\alpha \simeq 0.2$) means that the EC flux of a substance through the tissue is less than through bulk fluid for the same concentration gradient. The space available for build-up of extracellular substances is reduced by the same factor, however,

so Fick's second law is unaffected. Fick's laws for a truly EC substance for diffusion in one dimension (x) become:

$$J = \alpha D^* \frac{\partial C}{\partial x} \quad (1)$$

$$\frac{\partial C}{\partial t} = D^* \frac{\partial^2 C}{\partial x^2} \quad (2)$$

where J = flux per unit area of tissue, α = EC space fraction, $D^* = D/\lambda^2$, C = concentration of substance within the clefts. Note that essentially two distinct diffusion coefficients are defined by Fick's first and second laws: D_1 = flux per unit concentration gradient = αD^*; $D_2 = \frac{\partial C}{\partial t} / \frac{\partial^2 C}{\partial x^2} = D^*$. These are somewhat analogous to thermal conductivity and diffusivity in the theory of heat flow (e.g., Carslaw and Jaeger, 1959). The distinction vanishes in diffusion theory if concentrations are defined as the mass of substance per unit volume of tissue rather than as the true local concentration within the clefts. Both diffusion coefficients then reduce to D^*. But in many cases it is the true local concentration that is either measured (e.g., with ion-selective microelectrodes), or inferred (e.g., from the behavior of cells), or fixed (e.g., by equilibration with adjacent fluid). In these cases the macroscopic concentration, referred to unit volume of tissue, is an abstract quantity that could be inferred only with the help of measurements of EC space fraction. In work with radioactive tracers (e.g., Patlak and Fenstermacher, 1975; Pape and Katzmann, 1972), on the other hand, it is commonly the macroscopic concentration that is directly measured and the EC space fraction ceases to enter into diffusion calculations.

The distinction between the coefficients in Equations (1) and (2) above was overlooked by Lux and Neher (1973) when they examined the effect of K^+ flux on EC K^+ concentration around an iontophoretic point source (see Gardner-Medwin, 1978). A more rigorous examination of this experimental situation leads to different conclusions (Nicholson et al., 1979). In particular, it seems that K^+ ions behave as if they do not remain in EC space, while tetraethylammonium ions (TEA^+) behave as if they were purely extracellular. Experiments of this type, using EC markers, permit independent estimates of λ and of α to be made (see Nicholson, Chapter IV).

6. A Note on the Electrical Conductivity of Brain Tissue

The above conclusions have implications for cerebral impedance or conductivity measurements. Current flow is not restricted solely to the EC space, but the macroscopic DC conductivity of the tissue must be at least that attributable to ion movement within the EC space. If the ionic mobilities (or molecular diffusion coefficients) are the same within the clefts as within cerebrospinal fluid, then the EC component of the conductivity (σ) should be equal to the conductivity of cerebrospinal fluid (about 20 $mS \cdot cm^{-1}$) multiplied by α/λ^2. With $\lambda^2 = 2.5$ and $\alpha = 0.2$, this gives $\sigma = 1.6$ $mS \cdot cm^{-1}$. Measurements of cerebral conductivity vary with the site, direction, and the technique of measurement. Values of about 4 to 5 $mS \cdot cm^{-1}$ have been obtained with current flow perpendicular to the cortical and cerebellar surfaces (Van Harreveld, 1972; Nicholson and Freeman, 1975), about 7 to 12 $mS \cdot cm^{-1}$ in the direction of the principal fibers in white matter (Ranck and Bement, 1965; P.W. Nicholson, 1965) and in the cerebellum (Nicholson and Freeman, 1975), about 0.8 to 1.2 $mS \cdot cm^{-1}$ transverse to the fibers in white matter (Ranck and BeMent, 1965; P.W. Nicholson, 1965). The situation in which purely EC current flow is probably most nearly approximated is the last one, which also gives the closest agreement with the figure calculated above. It is probably fair to conclude that in the other situations much of the current flows through cells, as should be expected if there are cell processes extending in the direction of the current for distances longer than their electrical space constants. For the technique of current source-density analysis (Nicholson and Freeman, 1975), it is necessary in principle to know the true EC conductivity in each direction in the tissue; unfortunately, it is hard to conceive of a technique that could measure this in neural tissue without risk of distortion from the effects of currents flowing through cells.

7. Intracellular Diffusion

In situations where uptake of a substance occurs across cell membranes (see Part 3 above), a concentration gradient in EC space will lead to an intracellular gradient as well. Thus cytoplasmic diffusion may

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contribute to flux through the tissue. Small cells, however, may contribute to uptake but not significantly to diffusion, if they lack cell processes extending far in the direction of the concentration gradient. As is the case for the passage of electric current, there will be some characteristic length that processes must exceed in order to make a substantial contribution to intracellular diffusion. This characteristic length may be either smaller or greater than the electrical space constant of the cells, depending on the relative permeabilities of the membrane to the various ions involved.

The mathematical form of diffusion solutions with two compartments involved becomes more complex than that determined by Fick's laws and depends on geometrical and other factors. But in general the ratio flux/$(\partial C/\partial x)$ is increased and the ratio $(\frac{\partial C}{\partial t})/(\frac{\partial^2 C}{\partial x^2})$ is decreased by comparison with pure EC diffusion (Part 5 above). The contribution of this mechanism may in some circumstances be important, but it will always lag behind dispersal by EC diffusion because the first step involves the movement of solute across the membranes to build up an intracellular gradient.

8. Transport by Bulk Flow

Solutes are transported by bulk flow from the blood to the lymph within the interstitial spaces of many tissues. Bulk flow may also occur within cells (e.g., giant algal cells: Hope and Walker, 1975), and possibly as one of the mechanisms of axoplasmic transport (Grafstein, 1977). A flow of fluid out of the surfaces of brain tissue probably contributes about 30% of the rate of production of cerebrospinal fluid (Rapoport, 1976), amounting to about 10^{-6} ml of fluid per ml of brain per sec. The velocities required to account for this flow over the few mm between tissue sites and the ventricular surface are extremely small (of the order of a few nm·s^{-1}) and it is reasonable to expect that all but the largest and least diffusible molecules in the EC space would be able to equilibrate with the cerebrospinal fluid by diffusion much faster than they would be carried into it by bulk flow.

A significant amount of fluid may leave capillaries at the arterial side of the circulation and be driven by hydrostatic pressure through the interstitial spaces to be taken up again by vessels at the venous side

(see, e.g., Davson, 1970). Again, however, it is likely that the movement of solutes by EC diffusion over the short distances involved is very much faster than the contribution due to bulk flow.

One situation in which the transport of solutes by bulk flow does appear to be important is in the spread of cerebral edema due to disruption of the blood-brain barrier (Reulen et al., 1977). This is a slow process involving transport over several millimeters and constitutes a severe disturbance of the pressure and osmotic relations in the tissue.

9. Transport of Ions Associated with Current Flow: The Spatial Buffer Mechanism

When a disturbance of the brain cell microenvironment causes changes in the membrane potentials of cells, electric currents may be set up. These currents will occur when there is a gradient of membrane potential along the processes of a cell or between different cells that are electrically coupled. The current flow may serve to redistribute ions from one region of EC space to another: a process that has been called the "spatial buffer mechanism" as it was proposed by Orkand and coworkers (1966) as a means by which glial cells might contribute to K^+ dispersal.

Currents flowing through the membranes and fluid spaces of cells must flow in complete loops (Figure 9(7)). The redistribution of ions arises from the fact that the current in different segments of the loop may be carried in different proportions by different ions (i.e., the *transport numbers* are different). Thus, within the EC space, the current is carried predominantly by Na^+ and Cl^- because these are the majority ions. Where the current crosses the cell membranes and also within the cytoplasm, much of it is carried by K^+. Thus the effect of current flow on the EC space is to add K^+ where the current leaves the cells and to remove it where the current enters cells. The K^+-concentration changes are brought about partly by Na/K substitution and partly by build-up or depletion of KCl (the latter leading to osmotic shifts of water: the so-called "transport number effect" described by Barry and Hope (1969a,b).

Since the membrane potentials of cells, particularly of glial cells, are sensitive to the $[K^+]_o$, a gradient of $[K^+]_o$ will set up current loops. The direction of these current loops is such as to redistribute K^+

from regions where the EC concentration is elevated to regions where it is lower. The membranes need not be permeable to ions other than K^+, and indeed the mechanism works best if they are not. There is practically no lag in the development of flux (only a lag of the order of the membrane time constant), and with a localized build-up of $[K^+]_o$ there is essentially immediate commencement of K^+ transport over distances of the order of the electrical space constant of the cell processes involved. This is a mechanism that in the early stages is intrinsically much faster for dispersal than any sort of diffusion process. Recent work, outlined in the next chapter, suggests that, for steady fluxes over distances of the order of 100 μm or more, it is probably the predominant mechanism for K^+ migration through the tissue.