Review Article

Some Aspects of Fluid and Electrolyte Metabolism in the Brain

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AN UNDERSTANDING of nervous system function is obviously of prime importance to the anesthesiologist whose professional life is devoted to modifications of nervous system function. This review will deal with some aspects of fluid and electrolyte metabolism in the brain. The present review will be selective rather than exhaustive.**

Transport Processes in the Regulation of Electrolyte Composition

The processes involved in the regulation of the composition of intracellular fluid in any organ are part of the broad field of biological transport. Electrolyte metabolism in the brain depends on the nature of transport processes in this structure. It will therefore be useful to consider some general aspects of biological transport.

This area involves the study of the processes by which cells maintain an internal cellular environment which is distinct from the composition of the external environment and from that of plasma or extracellular fluid. Transport subserves the general function of providing adequate supplies of substrate, fluid, and electrolytes and the excretion of waste and surplus from the cell. For example, sodium transport in the red cell serves the function of regulation of red cell volume and optimum red cell composition.[69] In addition, there are specialized cells in which the transport of specific substances is required for the performance of functions which characterize the role of the cell in the body economy. For example, the transport of sodium across the membranes of

renal tubular cells serves to regulate Na^+ composition and represents a highly specialized function which not only regulates the internal composition of these cells but serves to regulate Na^+ composition in the entire animal.

Rapid changes of Na^+ and K^+ inside of neurons and nerves are an integral part of nerve impulse formation and transmission. *A priori*, it might be ancipiated that transport processes involving these ions are of major importance in central nervous system function.

The two general mechanisms which are involved in ion transport in the brain are: † (1) passive diffusion and (2) active transport.

Passive Diffusion. Passive diffusion refers to the statistical movement of molecules from an area of high concentration to an area of low concentration. This movement is energized by the intrinsic thermal energy of the molecules involved. Movement of substances into and out of the interior of the cell takes place across

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** For more complete and general reviews the reader is referred to two superb recent surveys in *The Handbook of Physiology.*[73, 76]

† Other well characterized transport mechanisms include: solvent drag, in which the penetrating substance is swept through aqueous pores of a membrane as a result of the bulk flow of water. The process is energized by the mechanism producing the flow of water, generally a difference in hydrostatic pressure or osmotic pressure across the membrane; facilitated diffusion, in which the combination of the transported species with a carrier molecule is located in the membrane. It is visualized that the combination of carrier and transported species takes place at one border of the membrane and the dissociation of the carrier-transported molecule takes place at the opposite border. Carrier-mediated transport may serve to increase the rate of transport and is referred to as facilitated diffusion. In this mode of transport the energy for transport is furnished by the chemical reaction between carrier and transported species and no external energy supply is required; and, pinocytosis, (cell drinking) in which there is the formation of small vacuoles in the cytoplasm of cells by phagocytosis-like entrapment of solution from extracellular fluid. This mechanism may explain the penetration of large molecules into the cell which might otherwise be incapable of penetrating the plasma membrane.

TABLE 1. A Comparison of Muscle and Brain Electrolytes in the Dog and the Dogfish Shark

Mammalian-Dog (41)	Plasma	Muscle	Brain
Na$^+$ mEq./liter H$_2$O	150	25	59
K$^+$ mEq./liter H$_2$O	5	96	95
Cl$^-$ mEq./liter H$_2$O	108	16*	34
Na$^+$ space		17%	40%
Cl$^-$ space		14%	32%

Shark (59)	Extra-cellular Fluid	Muscle	Brain
Na$^+$ mEq./liter H$_2$O	228	29.5	129
K$^+$ mEq./liter H$_2$O	3.4	187	162
Cl$^-$ mEq./liter H$_2$O	272	38*	99
Na$^+$ space		13%	56%
Cl$^-$ space		14%	36%

* Presumably most or all of the Cl$^-$ present in muscle is extracellular in location.

the plasma membrane. Thus, one important factor which determines the number of transported species which traverse the membrane per unit time is the molecular structure of this membrane. It is generally accepted that the usual membrane consists of a bimolecular lipoid layer with an adsorbed protein layer on both inner and outer surfaces.[17] Chemical species penetrating the membrane must, in essence, do so in solution. It follows that the higher the degree of lipoid solubility the greater will be the ease of penetration. Thus, the fundamental permeability of cell membranes to a wide variety of substances is a function of lipoid solubility. This fact is the basis of the well known Meyer-Overton law which relates lipoid solubility to increasing anesthetic effectiveness of a number of agents.[67] Other factors which determine the rate of passive diffusion include the anatomical nature of the membrane; the surface area available for diffusion; the temperature; the activity gradient of the species across the membrane (concentration gradient for uncharged species; electrochemical gradient for charged species); and the length of the diffusion path. In a number of biological systems the quantitative importance of these factors cannot be operationally evaluated and whether passive diffusion is the mode of diffusion cannot be clearly answered. In other systems, it is possible to decide whether the mode of transport is passive diffusion or not.

Active Transport. The movement of a species against an unfavorable electrochemical gradient or down a favorable gradient at a rate which cannot be accounted for by thermal diffusion alone is referred to as active transport. In order for such movements to occur free energy must be supplied to the system. This energy is made available by metabolic processes. In most forms of active transport the metabolic processes apparently reside in the plasma membrane itself. From this standpoint, the plasma membrane may be viewed as an organized subcellular unit or cell organelle with a more or less independent assortment of metabolic substrates, enzymes, energy sources, and regulatory processes permitting the generation and utilization of energy.

A whole host of substances in a wide variety of tissues have been regarded as requiring active transport for passage into or out of cells. Among the best characterized are the transport of the alkali metal ions, Na$^+$ and K$^+$, and it might be anticipated that active transport is involved in the regulation of brain composition with respect to these ions.

Steady-State Concentrations of Na$^+$, K$^+$, and Cl$^-$

The steady-state concentrations of substances inside of brain cells must ultimately be governed by transport rates. However, it is possible to determine concentrations of transported species under steady-state conditions. Such measurements may give insight into basic mechanisms responsible for the observed concentrations. It is therefore useful to discuss steady-state concentrations of Na$^+$, K$^+$, and Cl$^-$ in brain and consider the mechanisms involved in the establishment of these concentrations.

Concentrations of Na$^+$, K$^+$, and Cl$^-$ in the brain are summarized in table 1. Typical values from two species, one mammalian (dog) and one vertebrate (dogfish shark) are shown. The values for brain are contrasted with those for muscle which may be regarded as a tissue with a typical intracellular composition. In most tissues intracellular concentrations of Na$^+$ are low as compared with extracellular concentrations. For example, intracellular concentrations of Na$^+$ in mammalian muscle average approximately 25 mEq./liter muscle water,

whereas in extracellular water Na⁺ concentration averages approximately 150 mEq./liter. Intracellular concentrations of K^+ are relatively high (95 mEq./liter muscle water) whereas K^+ concentrations in extracellular fluid are low (5 mEq./liter). Cl^- concentrations are low and indeed there is evidence to suggest that this ion may be virtually excluded from most cells.[10] This virtual exclusion has led to the use of Cl^- space ‡ as an index of the volume of extracellular fluid.

The quantitative distribution of these ions in brain differs from this pattern. Na⁺ concentrations are considerably higher averaging approximately 60 mEq./liter brain water, in mammals. Thus, the Na⁺ space in brain is 40 per cent as compared with a Na⁺ space in muscle of approximately 20 per cent. Cl^- concentrations are considerably higher averaging 34 mEq./liter. Thus, the Cl^- space in brain averages approximately 35 per cent. The data in table 1 indicate that this electrolyte pattern in brain is also present in vertebrates as "primitive" as the shark and presumably constitutes a fundamental pattern within the central nervous system in many or most animals. K^+ concentrations on the other hand are approximately the same as in other tissues.

Not only are the concentrations of Na⁺ and Cl^- higher in brain water but these increased concentrations impose a significant problem. The osmotic activity of additional Na⁺ and Cl^- would seem to indicate that the total osmolality inside of brain would be significantly higher than inside of muscle. Since muscle has been reported to be in osmotic equilibrium with plasma [7] this finding would imply that brain is hyperosmotic with respect to plasma. On the other hand, direct measurements of brain osmolality suggest that this tissue is isosmotic with plasma.[50] Three explanations can explain this discrepancy.

(1) That the additional NaCl is located extracellulary. That is, that brain has a larger volume of extracellular fluid than peripheral tissues. § This is not likely. As will be discussed below, the bulk of evidence suggests that, if anything, the volume of extracellular fluid in brain is smaller and not larger than in most tissues.

(2) That some of these ions exist in brain water in a bound form and hence in an osmotically inactive form. The in vitro binding of Na⁺ and K^+ ions in aqueous suspensions of cephalin, a mixture of lipids which is found in high concentrations in nervous tissue, has been demonstrated by Christensen and Hastings.[8] It has been suggested that such binding also occurs in vivo and thus accounts for the apparent surplus of cation in brain tissue.[17] Such a mechanism would not account of course for the "excess" anion (Cl^-) found in brain.

(3) That the NaCl is heterogenously distributed among different cells. If some brain cells were rich in Na⁺ and Cl^- and others low, then depending on the absolute concentrations of these ions in the different cells and on the numbers of each cell type studied, analysis of a mixture of cells might give rise to concentrations that seemed high.

centrations in tissue should be emphasized. In the determination of intracellular ion concentration the investigator first analyzes the total ion concentration of the tissue. This total ion concentration includes the concentration of the ion inside of cellular water (intracellular concentration) and the concentration of the ion in extracellular water. To calculate the actual concentration of ion present in intracellular water, it is necessary to subtract the ion content present due to extracellular contamination from the total ion content. This degree of extracellular contamination may be determined by multiplying the volume of extracellular fluid by the extracellular concentration of the ion. This can be made clear by means of a typical example.

Total Na⁺ concentration in a given tissue = 75 mEq./liter tissue water.

Extracellular space: 20 per cent.

Na⁺ concentration in extracellular fluid: 150 mEq./liter extracellular water (obtained by measuring plasma Na⁺ concentration and assuming extracellular fluid is in Donnan equilibrium with plasma).

Na⁺ in tissue because of extracellular contamination $150 \times 0.20 = 30$ mEq./liter.

Na⁺ concentration, intracellular in tissue

$$= \frac{75 - 30}{1.0 - 0.20} = \frac{45}{0.80} = 56 \text{ mEq./liter}$$

tissue water. Thus, an estimate of the extracellular space is a critical determinant of the calculated value of intracellular electrolyte concentrations. The problem of the magnitude of extracellular space in the brain is particularly difficult and will be discussed extensively below.

‡ Cl^- space (percentage of total tissue water)

$$\frac{[Cl^-]_{tissue}}{[Cl^-]_{extracellular fluid}} \times 100.$$

§ The operational importance of measuring the volume of extracellular fluid in studying ion con-

There are two lines of evidence that suggest a heterogenous distribution of Na^+ and Cl^- as an explanation for the high concentrations of these ions in "mixed" brain tissue.

(1) Analysis of gliomas which are more or less pure collections of glial cells suggest that glial cells are relatively rich in Na^+ as compared with K^+.[28]

(2) There is conclusive evidence that some of the Cl^- present in brain tissue is intracellular in location.[30] Thus, at least some cells in the brain must be high Cl^- cells.

The steady-state distribution of electrolytes in the brain may be summarized as follows:

Brain cells on a statistical basis contain higher Na^+ and Cl^- concentration than do other cells. K^+ concentrations are similar to those of other cells. This pattern is present in many different species. Although the basis of this unusual pattern of distribution is not known, one explanation that is consistent with present evidence is that some cells (glia) are rich in Na^+ and Cl^- and others are poor in these ions.

Relation of Brain Electrolytes to Changes in Plasma Electrolytes

Changes in the electrolyte composition of plasma generally produce marked changes in the intracellular composition of most cells. There are two aspects of this process.

One aspect concerns the rapidity of electrolyte exchange between plasma and cell water. This aspect can be approached by adding radioactive isotopic ionic species to plasma in tracer amounts and measuring the rate of equilibration of these isotopes in cellular water. The second aspect concerns the effect of modification of plasma electrolyte concentrations on the electrolyte composition of intracellular water. This aspect can be studied by modifying plasma composition and noting the effect of this modification on steady-state electrolyte composition of brain. Both of these aspects are different in brain than in most peripheral tissues like muscle. Following the injection of radio-labelled Na^+, K^+, or Cl^- into plasma the rate of equilibration of these ions in brain tissue is quite slow[43, 44, 60] as compared with muscle or other tissues. This slowness of penetration has been attributed to the function of the blood-brain barrier (see below) and in-

deed labelled ions injected into the subarachnoid space penetrate brain rapidly.[53]

Changes in brain electrolytes in response to more chronic changes in plasma electrolyte concentrations may be summarized as follows:

The intravenous administration of hypertonic NaCl produces marked and prolonged dehydration of the brain suggesting that the rise in plasma NaCl concentration is not reflected by a corresponding rise in brain NaCl content.[64] Marked reduction in plasma Cl^- concentrations by means of perfusion with Cl^- free fluid produces only a minor decrease in brain Cl^-.[2]

Lowering plasma K^+ by the injection of adrenal steroids or increasing plasma K^+ by adrenalectomy does not produce corresponding changes in brain K^+.[12, 34, 73] Thus, it is clear that brain tissue is not in simple diffusion equilibrium with plasma and that changes in brain electrolyte composition occur only slowly, following changes in plasma electrolyte composition.

Changes in Electrolyte Distribution as an Intimate Part of Neural Function

Steady-state measurements of electrolyte concentrations in mixtures of brain obscure the transient shifts that occur. This is unfortunate since shifts in electrolyte constitute the very essence of neuronal function. The pattern of shift has been outlined by a number of workers notably Hodgkin[25] and Huxley.[27] Much of this work is based on analyses of single nerve fibers. The intracellular concentrations of Na^+ and K^+ may be analyzed directly as in the case of the squid giant axon where the axoplasm can be extruded and analyzed. The extracellular concentration of these ions is that of the medium in which the fiber is maintained. Manipulating the extracellular composition reveals that the resting transmembrane potential is related to the concentration gradient for K^+, $[K^+]_i/[K^+]_e$. During the passage of the nerve impulse (action potential) there is a transient but marked increase in Na^+ permeability and a concomitant decrease in K^+ permeability so that the Na^+ concentration gradient $[Na^+]_i/[Na^+]_e$ determines the magnitude of recorded potential. This work has been elegantly quantitated by direct measurements of Na^+ and K^+ fluxes across nerve mem-

branes. It has also been demonstrated that the inequality of ion concentrations across the nerve is maintained by the active transport of Na⁺ from the inside of the nerve to the outside.

The operation of these processes may well explain the relatively high O_2 consumption of brain. Approximately 25 per cent of cardiac output and 25 per cent of total O_2 consumption is used by the brain, which represents only 2 per cent of total body mass.[29] Why this structure should have high O_2 (energy) requirements is not obvious since it performs no external mechanical work and is not intrinsically a secretory organ. This high O_2 consumption is present during sleep and is not modified by increased mental activity.[45, 65] The transport of ions against electrochemical gradients is an energy requiring process. It appears probable that the major work of the brain consists of ion transport which in turn explains the high O_2 requirement of brain tissue. This viewpoint is supported by the demonstration that the in vitro O_2 consumption of brain slices can be increased by electrical pulsing.[47] ¶

A problem posed by the involvement of ions in nerve impulses concerns the media in which the necessary movement of ions takes place. Since there is only slow penetration of ions from plasma, there must be some specialized variety of extracellular fluid (in a sense a specialized variety of microenvironment), bathing brain cells, which is involved in electrolyte ex-

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¶ As outlined by Anderson and Ussing [8] the work required in active transport may be divided into three parts. The work required to overcome (1) the activity (concentration) gradient; (2) the electrical potential gradient; and (3) internal resistance of the membrane. Neglecting 3, the work, in calories, required to transport one mole of substance from one side of a membrane to the other would be

$$W = 0.239 \ RT \ \ln_e C_2/C_1 + ZF \ (\psi_2 - \psi_1)$$

where

R = gas constant
T = absolute temperature
C_2/C_1 = activity gradient ratio
Z = charge (plus, minus, or zero)
F = Faraday
$\psi_2 - \psi_1$ = electrical potential gradient

In the case of brain the majority of this energy must come from oxidative dependent metabolism, hence the high O_2 requirement of this structure.

changes in vivo. Thus, the problem of the nature and magnitude of brain extracellular space is an important aspect of the mechanism which govern neural function itself.

Brain Extracellular Space and Blood-Brain Barrier

The conceptual basis of an extracellular space is founded on three assumptions:

(1) That there is a substantial volume of fluid in tissues which is located outside of cells.

(2) That a portion of this fluid is formed as an ultrafiltrate or dialysate of plasma. This segment of extracellular fluid is the interstitial fluid.

(3) That this fluid abuts on cell membranes and is the median by which intracellular-extracellular exchanges occur.

Classically, measurements of the volume of extracellular fluid have been based on the use of substances of suitable sizes or chemical properties which are capable of freely penetrating capillary walls and distributing themselves equally throughout extracellular fluid. These substances should be unable to penetrate plasma cell membranes and therefore be excluded from intracellular water. The degree of dilution of such substances can therefore be used to calculate the volume of extracellular fluid. The criteria for establishing the appropriateness of various substances capable of being retained only in extracellular water have never been rigorously established. In general, two types of substances have been employed: (1) more or less metabolically inert carbohydrates such as sucrose (MW = 300) and inulin (MW = 5000) which can penetrate capillaries but are, hopefully, too large to penetrate cell membranes; (2) anions like $SO_4^=$, Cl^- and SCN^- which are presumably excluded from cell water because of an unfavorable electrical gradient. This general approach has been considered acceptable because in most tissues the spaces defined by the various substances have been more or less equal. Thus, the Cl^- space, SCN^- space, inulin space, and sucrose space of muscle are approximately 20 per cent and this figure is accepted as the extent of the functional extracellular space of this tissue.

One could visualize a three compartment system as being present in tissues. There is

the v~~ascular compartment~~ment in diffusion equilibrium with a second compartment, ~~interstitial fluid~~. The interstitial fluid in turn bathes cell surface and communicates with a third compartment, the ~~fluid within cell membranes~~es.

Until recently, this picture of a discrete and easily defined extracellular space containing extracellular fluid (ECF) was accepted for brain and the nervous system. Light microscopy indicated large spaces surrounding neural elements which were accepted as the anatomical counterpart of a functional extracellular space. With the advent of electron microscopy this picture changed. Numerous studies [14, 15, 41, 75] showed that ~~there were no substantial extracellular spaces in brain~~. The space between neurons was filled almost completely with glial cells. The plasma membranes of the cells were separated by an interspace only 100 to 150 Ångstroms units wide, occupied by an amorphous substance of relatively low density. Estimation of the volume occupied by such spaces amounted to only 4–5 per cent of the total volume of the brain.[26] As a result of this picture it was concluded by most electron microscopists that the large spaces seen on light microscopy were artifacts and that a true extracellular space in brain, if it existed, must be quite small.

Examination of the volume of brain extracellular space by classical physiological techniques does not succeed in resolving the problem. Measurements of sucrose, inulin, and $SO_4^=$ space in the brain following intravascular injection of these substances showed spaces of approximately 5 per cent.[4, 30, 57] This estimate agrees rather well with the estimates seen on electron microscopy. However, there are two possible interpretations of such data:

(1) That brain ECF is actually approximately 5 per cent.

(2) That measured spaces by these substances are inaccurate because there is no free access of these substances to the brain. The inability of these substances to freely penetrate brain was attributed to the blood-brain barrier. Thus, implicit in the problem of volume of brain ECF is the problem of the nature and location of the blood-brain barrier (see below).

Attempted measurements of brain ECF by the determination of Cl⁻ space likewise have lead to no clearcut conclusion. Measurements of endogenous Cl⁻ space,

$$\frac{[Cl^-]_{brain\ tissue}}{[Cl^-]_{in\ plasma}} \times 100$$

give rise to values of approximately 35 per cent.[30] It appears inconceivable that brain extracellular space could be of this magnitude. A priori, the possibility that a significant amount of brain Cl⁻ was intracellular, in location, appeared reasonable. Recent studies have established indeed that a significant portion of brain Cl⁻ is intracellular in location and that measurements of brain Cl⁻ space using endogenous Cl⁻ are not an acceptable estimate of the volume of extracellular space.[30] Measurements of brain Cl⁻ space by using exogenously administered Cl⁻ show that the penetration of brain by this ion follows a complex kinetic pattern.[74] During the early phase of penetration the radio-labelled Cl⁻ equilibrates with only a small volume of brain water. This can be interpreted as demonstrating only a small volume of extracellular fluid or alternately as the exclusion of Cl⁻ by the blood-brain barrier. As time continues, an increasing amount of the labelled Cl⁻ penetrates the brain, presumably as a result of equilibration with the intracellular pool of Cl⁻.

The use of classical physiologic techniques give rise to data which can either be interpreted as being consistent with a relatively small volume of brain extracellular fluid or alternately with the presence of a distinct barrier to the penetration of indicator substances so that the apparent extracellular space is small. Thus, the problem has not been settled in definite fashion by such studies.

Another physiologic approach has been to inject indicator substances directly into the subarachnoid space to bypass the blood-brain barrier and so measure the volume of extracellular fluid. In the case of inulin, such measurements give rise to a space

$$\frac{[Inulin]\ brain}{[Inulin]\ CSF} \times 100$$

of approximately 10 per cent.[56] Such studies clearly demonstrate that inulin is capable of equilibrating with a fluid space of considerable volume in the brain. They have been inter-

preted as showing an extracellular space of approximately 10 per cent. In reality this study has succeeded in quantitating the volume of cerebrospinal fluid in the brain. Unanswered is the question as to whether spinal fluid is the functional extracellular fluid of the brain. It is certainly clear that spinal fluid is extracellular in location. However, whether the two other characteristics of extracellular fluid in peripheral tissue apply: (a) directly formed from plasma as a dialysate, (b) is the direct medium of exchange for brain cells is not clear. In regard to a, it is certain that the composition of CSF cannot be explained as a simple dialysate of plasma. A number of studies have clearly indicated that the concentration of a number of constituents of CSF including Na^+, Cl^- and Mg^{++} [55] cannot be explained on the basis of simple passive diffusion. This implies that the composition of CSF is regulated by the metabolic activity of those cells involved in the formation of CSF. In this sense, then, the local environment of the brain would be controlled by the activity of the cells which manufacture CSF. This would be analogous to the regulation of the composition of peripheral extracellular fluid by renal activity.

A more difficult question to answer is whether CSF is in such intimate relation to brain cells that it is capable of engaging in intracellular fluid and electrolyte exchange. This would constitute no serious problem for brain cells fairly superficial in position and in the walls of the ventricular system. Just how much exchange could occur between spinal fluid and cells deep in the brain is difficult to say. In any case, there are no convincing data to accept or reject the hypothesis that CSF is essentially the functional ECF of the brain.

One is therefore left with the uncomfortable problem of a tissue which engages in brisk electrolyte exchange and no apparent volume of extracellular fluid with which such exchanges can occur.

Another area which might serve as the counterpart of extracellular fluid in the brain is the mass of glial cells. This viewpoint has been vigorously championed by Lumsden [40] and DeRobertis.[16] The evidence that glial cells constitute the functional extracellular fluid of the brain is of several varieties:

(1) Electron microscopy reveals that glial cells occupy a unique position with respect to brain capillaries. There is a rich proliferation of processes originating in the cell body of glia and investing brain capillaries (glial foot processes). Glial cell processes likewise richly invest neurons. Thus, anatomically, the glia would appear to be interposed between the capillary and the neuron,[16] filling all or most of the intervening space between vascular elements and neurons.

(2) Experimental conditions leading to increases in brain water content (cerebral edema), lead to a swelling of glial cells without a corresponding visible distension of any potential extracellular space.[19, 68] Thus, in such circumstances the glia would appear to function clearly as an extracellular space.

(3) The probable electrolyte composition of glia previously commented upon as being relatively high in Na^+ and Cl^- resembles that of extracellular fluid and not intracellular fluid,[28] and is consistent with a probable role as extracellular fluid.

(4) Glia cells are metabolically active. Coggeshall and Fawcett [9] have shown the presence of large amounts of glycogen and scattered fat droplets within the cytoplasm. These cells likewise contain a great number of mitochondria and a rich supply of endoplasmic reticulum. Thus, these cells possess the structural basis for the energy generation necessary for transport processes.

(5) Glial cells show evidence of extensive spread of electrical current from one cell to another.[33] Thus, a localized disturbance is capable of being transmitted widely to a central pool, obviously a characteristic true of extracellular fluid.

(6) Glial cells in $vitro$ have been shown to manifest pinocytosis.[23] Glial cells have been shown to be capable of taking up fluorescein-labelled protein [31] and ferritin.[61] Thus they are capable of transporting relatively large molecules.

(7) The assumption that glia serve as the functional "extracellular" fluid in the brain would permit a unitary explanation for the blood-brain barrier (see below).

There have been two major objections to the ready acceptance of this concept:

One objection is the ability of extracellu-

lar markers like inulin, injected directly into the subarachnoid space, to penetrate brain tissue.[56] Since these substances must be diffusing into some type of fluid, it is argued that this fluid is brain extracellular fluid. It is clear of course that the fluid receiving these substances is CSF and this objection reduces itself to the problem of whether CSF can be regarded as the functional ECF of the brain.

The second objection stems from electrical impedance measurements of brain tissue. The impedance of a tissue is mainly a function of the extracellular electrolytes. Cell membranes generally have high electrical resistance and a measuring current passed through an organ or a cellular suspension is carried almost exclusively by extracellular electrolytes. Impedance determinations of rabbit cortex were performed by van Harreveld, Hooper, and Cusick.[71] The relatively low values found and the increase in impedance during the administration of 50 per cent glucose and during hypoxia were interpreted as indicating that there was a large volume of Na$^+$ and Cl$^-$ ions not enclosed by high resistance cell membranes; thus indicating that brain must have a substantial volume of extracellular fluid. A priori, one might anticipate that the electrical properties of glial membranes might be quite different from those of other membranes. In view of the high concentration of Na$^+$ and Cl$^-$ in these cells, it appeared possible that such membranes might have low electrical resistance. Recent measurements by Hild and Tasaki [23] have confirmed this possibility. Glial cells from young cat and rat cerebella were cultivated in vitro. The membranous resistance of the glia was measured by means of intracellular microelectrodes and was found to be rather low. Thus, the special electrical properties of the glia are qualitatively consistent with the measurements of impedance in the intact brain and such measurements do not rule out, but actually enhance the possibility of glia serving as "extracellular fluid."

To date, the critical experiment testing this possibility has not been devised. In the absence of such a critical experiment, this hypothesis explains a wide variety of experimental findings and holds great promise.

Intercellular Clefts. Early electron microscopic studies of the brain suggested that glial cells filled virtually all of the space between neurons. The cell membranes were separated by a interspace measuring 150 to 200 Ångstroms in width; even these seemed to be filled by an amorphous substance of low electron density. However, as pointed out by Coggeshall and Fawcett,[9] whether there was *no* space between cells or whether there was a real space, albeit limited in extent, depended upon an interpretation of fine details of electron-micrographs of a complicated structure. Recent work suggests that in reality there are narrow clefts between brain cells and that these clefts are occupied by a thin gel. The detailed appearance of these clefts can be accentuated by appropriate electron-micrographic technique. They also become widened by exposure of the tissue to hypertonic solutions.[58] In the kidney, it has been shown that molecules as large as hemoglobin can diffuse along the 150 Ångstrom spaces between cells.[48] Similarly, ferricyanide has been found to diffuse freely into the intercellular clefts in toad retinas.[35] The total volume occupied by the intercellular clefts has been estimated at 4–5 per cent of total volume, a value, which possibly on a coincidental basis, agrees with physiological estimates of sucrose and inulin space in the brain.

The exact role played by the intercellular clefts, and their gel-like matrix, in water and electrolyte metabolism is not clear but it appears possible that some transport occurs in this area and that it may at least, in a limited sense, constitute part of the functional ECF of the brain.

The Blood-Brain Barrier. The origin of the concept of a blood-brain barrier was the discovery by Ehrlich (1885) that intravenous injection of the dye coerulin A did not stain the brain, while it stained other tissues. Subsequently up to the 1900's a similar exclusion from the brain following intravascular injection of tetanus toxin,[63] bile,[6] and sodium ferricyanide [38] was shown. The classical studies in this respect were those of Goldmann [21] who used the dye trypan blue. Following intravenous injection, *in vivo*, there was staining of all tissues except brain. Following intrathecal injection, the brain became blue-stained and the animals developsd seizures and paralysis. Following death, or damage to

brain tissue, however, there is staining of the brain by dyes such as trypan blue. Studies with various electrolytes and nonelectrolytes previously alluded to, likewise showed a significant lag before penetration into the brain. The sum total of this unusual property of transport from blood to brain is spoken of as the "blood-brain barrier."

One important aspect of this problem is the determination of anatomical location of the barrier to the transport of the various substances.

A number of sites have been proposed. Goldmann [21] originally suggested that the choroid plexus was the structure involved and assumed that all substances must pass through the choroid plexus in order to penetrate brain. However, a number of different experimental approaches have demonstrated that neither of these suppositions is correct.[70]

Another site suggested for the location of the blood-brain barrier are the capillaries of the brain. It has been suggested that the endothelium of cerebral capillaries form a continuous layer, without the fenestrations, seen in other capillary beds.[46] Supposedly, then, this lack of fenestration might account for the unusual barrier present in the brain. Other workers have suggested that the basement membrane of brain capillaries forms a continuous layer, and hence is the site of the blood-brain barrier.[1] However, there is no clear evidence that the basic structure of cerebral capillaries is different from other capillaries, except for the close apposition of glial processes. It is of interest that there are several areas in the central nervous system which apparently lack the characteristics of the blood-brain barrier. These areas include the area postrema, some nuclei of the chiasmatic infundibilar region of the hypothalamus, the neurohypophysis and pineal body. These regions show dye-staining after intravascular injection and are also permeable to large proteins and some ions. The structure of the capillaries in these regions does not appear to differ from capillaries in other regions of the brain but does lack the vascular processes originating in the glia.[16]

Another proposed site of the blood-brain barrier is the perivascular pial-glial membrane. This membrane was considered as made up of pia which followed cerebral vessels from the surface of the brain into the depth of the brain.[22] Electronmicroscopy has failed to verify the existence of such a structure, distinct from the vascular glial foot processes.

Finally, it has been suggested that the blood-brain barrier consists of the cell membranes of glia cells.[16] This theory has much to recommend it. The ability of lipoid soluble substances to penetrate the blood-brain barrier resembles the ability of these substances to penetrate cell membranes generally. The location of the barrier at the glial site is certainly consistent with the results of electron microscopy. The absence of barrier in areas lacking glial foot processes is strong support for such a view. Finally, this theory places, on a unitary basis, not only the blood-brain barrier but the special electrolyte composition of the brain, and the special features of electrolyte metabolism in this area. None of these facts incontrovertibly establishes the glia as the site of the blood-brain barrier, but this must be considered as a reasonable possibility.

H+ and HCO₃⁻ Metabolism. The metabolism of the ions in the brain is of special interest to the anesthesiologist because he is often confronted with abnormalities of acid-base metabolism and also because, under normal circumstances, the regulation of ventilation appears to depend to some extent on acid-base changes involving the central nervous system.

In common with most cells, brain water is substantially more acid than extracellular fluid. Mean brain pH measured in brain homogenates averages 6.9 to 7.0 pH units at a plasma pH about 7.4, so that there is a gradient of approximately 0.4 to 0.5 pH units.[30] The mechanism of this gradient is not clear but it seems unlikely that it reflects a simple Gibbs-Donnan equilibrium since the permeability of the brain to H^+ and HCO_3^- seems to be low. Not only is brain pH significantly more acid than plasma, but the pH of this organ responds differently to changes in pH produced by modifying extracellular pH by changes in P_{CO_2}, as compared to changes produced by mineral acid, HCO_3^-, and TRIS administration.[30] Changes in pH produced by manipulating extracellular P_{CO_2} are rapidly reflected by parallel changes in brain pH whereas

changes produced by the other three chemical species are reflected only slowly.

One possible explanation for this difference is that CO_2 is highly lipoid soluble whereas the other three species are relatively lipoid insoluble. It is also possible that the relatively poor permeability of brain for the three species is related to some form of active transport process or that this difference reflects a difference in buffering capacity in the brain as compared with blood.

The special permeability characteristics of brain have been known to be of probable importance in two clinical situations. In patients with chronic pulmonary disease and hypercapnia, rapid lowering of P_{CO_2} by mechanical ventilation may precipitate severe cerebral intracellular alkalosis and the development of twitching, focal seizures, coma, and death. The development of cerebral alkalosis under these circumstances presumably occurs because, with mechanical hyperventilation, cerebral P_{CO_2} falls rapidly whereas brain HCO_3^- falls slowly leading to cerebral intracellular alkalosis.[62]

A second clinical implication is the use of Tris as a buffer to combat acidosis. One theoretical advantage claimed for this substance is that it is capable of rapidly penetrating cell membranes, hence is useful in the treatment of intracellular acidosis.[51] Although this may be true of most cell membranes, it does not appear to be true of the brain.[30]

The ability of changes in acid-base parameters to evoke changes in the volume of ventilation has long been recognized and there is an extensive literature concerning this relation.[11, 52] The exact sites at which these changes provoke altered neural activity, the quantitative relationship between the various stimuli, and the exact mechanisms by which the changes in acid-base parameters are translated into increased ventilation, are not clear.

In recent years, two areas have emerged as the possible locus of the central chemical regulation of ventilation. One such area concerns the cerebrospinal fluid. Leusen demonstrated that perfusion of cerebral ventricles in anesthetized dogs with various acid-base active substances produced substantial changes in the volume of ventilation.[36, 37] This work has been extended by a number of workers, including Loeschcke,[39] Mitchell and Severinghaus,[40] and Pappenheimer.[54] Loeschcke and Mitchell and Severinghaus have interpreted their results as indicating that the neuronal cells responsible for such responses are located not in the classical respiratory medullary center, but 5–6 mm. away, on the ventro-lateral surface of the medulla in the superficial layers of brain. These workers have suggested that the specific stimulus to these cells is the H^+ concentration of cerebrospinal fluid or the immediate extracellular environment of the receptors which has a composition closely related to that of CSF. Local application of acetylcholine or nicotine to these areas produces hyperpnea, while cyanide and procaine produce respiratory depression. Since this approach emphasizes the importance of CSF in the regulation of ventilation, Severinghaus and Mitchell[49] were led to consider the possibility of regulation of CSF compositoin with respect to H^+ and HCO_3^-. A number of workers, including Davson[13] and Pappenheimer,[54] had shown that the composition of CSF with respect to a number of substances including organic dyes and various ions could not be explained on the basis of formation of CSF as a simple dialysate of plasma. Severinghaus and Mitchell measured the potential difference between blood and CSF and found a value of approximately 4 millivolts, the CSF being electrically positive with respect to the blood. On this basis, they concluded that HCO_3^- ion is actively transported into spinal fluid, since its steady-state concentration gradient is out of keeping with the electrical gradient. They suggested that the changes in HCO_3^- concentration in CSF during ascent to high altitudes and the hyperventilation seen in mechanically hyperventilated patients were reflections of the active regulation of HCO_3^- of CSF. They emphasized the importance of CSF HCO_3^- concentrations in the regulation of the volume of ventilation.[49]

Although these studies are intriguing, there are a number of objections to these concepts:

(1) The hypothetical superficial medullary chemoreceptors have not been identified anatomically or histologically.

(2) The interpretation of the meaning of potential difference measurements between

two fluids (blood and CSF) which are separated by several cell membranes, is obscure. Thus, the quantitative proof for an unfavorable electrochemical gradient is not complete.

(3) Regulation of CSF composition must be performed by cells. On the basis of available data it would be difficult to establish to what extent changes in CSF composition are responsible for changes in ventilation, and to what extent changes in CSF composition merely reflect some more fundamentally important changes occurring in the composition of central nervous system cells which are really the cells involved in the regulation of ventilation. In this respect, Pappenheimer et al.[54] recently studied the respiratory response to inhaled CO_2 during perfusions of cerebral ventricles with solutions of varying acid-base composition in unanesthetized goats. Alveolar ventilation increased significantly when HCO_3^- concentrations in CSF or pH of CSF were reduced at a normal constant P_{CO_2}. Large changes in ventilation in response to inhaled CO_2 occurred even when H^+ in cranial CSF was maintained constant. They interpreted these data as indicating that alveolar ventilation was a linear function of H^+ in tissue fluid located two-thirds to three-fourths of the distance along the functional concentration gradient of HCO_3^- between CSF and blood. They thus concluded that the basic premise of Winterstein[72] and of Gesell[20] that central chemical control of breathing was mediated by changes in the H^+ of fluid in immediate contact with respiratory neurons is correct. Although some of the conclusions of this study depend on a number of assumptions (albeit reasonable) the experimental results would seem to be incompatible with a simple straight line regulation of ventilation as a function of changes in CSF composition.

It seems that exact knowledge of the chemical regulation of ventilation will await the development of methods which permit accurate determinations of acid-base parameters in the specific neural cells responsible for regulation. The recent revival of interest in this area should result in a deeper understanding of the mechanisms involved.

Water Metabolism. The permeability of most cells to water is generally high. This high permeability is shared by the brain.

Bering[5] showed that intravenously injected deuterium-labelled water rapidly entered all areas of the brain and CSF, reaching equilibrium with the blood in approximately 20 minutes. This rapid permeability insures that the activity of water in plasma and brain must be equal at all times except for relatively brief transients. This fact has several important implications.

(1) Whole body water deficits (dehydration) will be accompanied by dehydration of brain.

(2) Cerebral water loss may be produced by the intravascular injection of substances which equilibrate more slowly with brain than does water.

Under these circumstances the fluid in the blood vessels of the brain will be relatively hypertonic as compared with brain water (smaller water activity in blood). Water will then flow down its activity gradient from brain into blood vessels and cerebral water content will be decreased. Perfusion of hypertonic solutions into a single carotid artery is capable of producing unilateral cerebral dehydration on the side of the hypertonic infusion.[64] This process has been demonstrated by the intravenous injection of urea[82] and is the mechanism which underlies the use of hypertonic glucose or mannitol in the therapy of cerebral edema. The less permeable a given substance is, the longer will be the time interval before the re-establishment of osmotic equilibrium. Of course, given a sufficiently diffusible substance and given sufficient time, osmotic equilibrium will again be re-established.

General Comments

Two general comments may be appropriate in assessing the material which has been presented in this paper.

One comment concerns the local autonomy that appears to characterize the regulation of electrolyte metabolism in the brain. This appears to be only one example of the characteristics of many aspects of cerebral metabolism. Other examples include the lack of insulin requirement for glucose metabolism in the brain[24] and the relative unresponsiveness of cerebral tissue to excess of thyroid hormone.[66] Such autonomy presumably has important value for survival. Marked swings in

the environment of metabolism of peripheral tissues are usually compatible with life. Such changes in the brain may result in the death of the entire organism. It is not surprising that mechanisms exist which buffer the brain against rapid changes in composition occurring in the blood or peripheral tissues.

The second comment concerns the necessity for caution in the interpretation of data obtained in the brain in terms of the simple physical-chemistry of solutions. This caution is required because of the marked heterogeneity of the brain.

First of all, the brain does not represent a single functional structure. The brain is in reality a collection of organs. Thus, there may be widespread variation in electrolyte metabolism depending on which part of the brain (which organ) is being considered. Studies of the intact brain may mask changes occurring in local regions of the brain with highly specialized functions.

Secondly, the brain is a collection of different cell types, each of which may have special characteristics in regard to electrolyte metabolism and special functions. Studies involving the entire mass of cells which give overall statistical values for composition may obscure important specific functions of one cell type or another.

Finally, as with all cells, the interior of the brain cell is quite heterogenous. There are organized subcellular units such as mitochondria, Golgi apparatus, microsomes, etc., which may have distinct properties and composition. The presence of macromolecules in various states of aggregation and dispersion likewise contribute to heterogeneity. There may be intracellular binding of water and electrolytes so that concentrations of these substances do not indicate true chemical activity. There may be distinct compartmentalization of cell constituents which may not be apparent from measurements of cell homogenates or brei. The most basic assumption of all, namely that intracellular electrolytes are in an essentially aqueous solution and show the *in vitro* properties of electrolytes in aqueous solution, may not be true in the brain. Rapid progress can be expected with the development of new methods and new concepts appropriate for dealing with such heterogenous systems.

Summary

The survival of the intact organism requires a central nervous system which is sheltered from the perturbations which occur in peripheral tissues and plasma. On the other hand, rapid and extensive ion transfer between neurons and their microenvironment are required for neuronal function. Under these circumstances, it is not surprising that complicated and unique mechanisms exist in the brain for the regulation of electrolyte metabolism on a local basis. Electrolyte distribution in the brain, the volume of brain extracellular space, the blood-brain barrier, and the function of glial cells are different aspects of a common solution to the general problem of stability in the face of continual change.

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