MECHANISMS OF DRUG ABSORPTION AND EXCRETION

PASSAGE OF DRUGS IN AND OUT OF THE CENTRAL NERVOUS SYSTEM[1,2]

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Introduction

Within the past few years, there has been a striking expansion of the understanding of the processes which govern the exchange of drugs between blood and the tissues and fluids of the central nervous system.[3] The mystical quality of the euphonic blood-brain barrier has been destroyed by well-planned experiments. A surprising number of previously speculative notions now rest on supporting experimental data. The methodology and concepts developed in the study of ion and drug movement in the kidney and across other biological membranes, have been effectively utilized in the study of the exchanges between blood, cerebrospinal fluid, and brain.

The plan of this review is to deal with methodological considerations and then to explore what might be called the pharmacological anatomy of the CNS including the formation and flow of the CSF. Understanding of the newer concepts in these areas is basic to the primary portion of this review which is a consideration of the exchange of compounds between blood, CSF, and brain. A concluding section will explore the implications of the above in a few clinical situations.

Methodology

Two areas will be discussed: first methodological problems, and, second, the potentialities of new techniques.

The questions to be answered by a typical experiment concern the extent, rapidity, and mechanism of the exchange of a compound between blood, CSF, and brain. In general, there are three approaches. The first two are *in vivo* techniques. The compound is added to the blood and its entry into the CSF and brain is studied, or the compound is added to the CSF and the loss of drug from CSF, or entry into brain and blood is studied. The third is the use of *in vitro* methods.

Of the various mechanisms of exchange which can occur, the first and

[1] The survey of the literature pertaining to this review was concluded in Aug. 1961.

[2] The following abbreviations will be used: CNS (central nervous system); CSF (cerebrospinal fluid); ECF (extracellular fluid).

[3] A number of reviews covering somewhat different aspects of this have been published (1 to 5).

simplest is passive diffusion,[4] in which a compound moves from one compartment into another down an electrochemical gradient. Passive diffusion must be ruled out before active mechanisms are invoked. Concentration ratios between plasma, CSF, or brain that differ markedly from unity are consistent with exchange by passive diffusion. A compound bound to plasma proteins[5] will behave with the characteristics of the drug-protein complex, and thus the apparent exclusion of an extensively bound drug from a protein-free compartment (CSF) compared to a protein-rich compartment (plasma) will reflect the exclusion of the drug-protein complex, and not necessarily the drug itself, Examples of this include the failure of trypan blue (6) and bilirubin (see later discussion) to enter brain and CSF, and the effect of sulfinpyrazone on increasing the entry of extensively protein-bound sulfonamides into brain by displacement of sulfonamide binding (7).

Two measurements describe the exchange of a drug by passive diffusion between blood and CSF (or brain). These are the rate constant and the final or steady-state ratio of the drug concentration in CSF water to drug in plasma water (drug not bound to serum protein) (5, 8, 9). If a constant plasma concentration is maintained, the estimation of these values is facilitated. When it is technically difficult to maintain the plasma concentration at a constant value, the rate constant and the steady-state ratio can be estimated if the CSF : plasma concentration ratio is followed until it becomes constant (5). Incomplete study of the time course of the exchange of a compound, however, can lead to erroneous estimates.

Misleading results also may be obtained when metabolism of the compound studied occurs on one side of the membrane, particularly if the method measures both metabolic product and original compound (2).

Other factors which result in steady-state ratios different from unity are the existance of a H^+ ion or potential difference across a membrane, and this will be considered in later discussion.

Until recently, one of the major problems in the study of drug exchange between blood, CSF and brain has been the inability to control the concentration of the drug in compartments other than blood. The development of a method of perfusion of the cerebral ventricles and cerebral subarachnoid spaces with synthetic CSF by Feldberg and co-workers (10, 11) and Pappenheimer and co-workers (12), has been a major advance. Pappenheimer & Heisey (13) have shown that this method estimates entry and exit rates of compounds into and out of the CSF, rate of formation of CSF, and the effect

[4] With the advent of a proven active transport system in the choroid plexus (see following discussion), it has become tempting to ascribe to active transport any instances in which the CSF or brain: plasma ratio of a compound deviates from unity. It is important to recognize that exchange by passive diffusion can and does result in cerebrospinal fluid or brain: plasma ratios greater or less than 1.

[5] In this review the phrase "extensively bound" to plasma protein means that 70 per cent or more of the compound is reversibly bound to protein at normal plasma concentrations; "slightly bound" will imply binding of 30 per cent or less.

of various compounds on CSF, formation. In this system cannulae are implanted into one lateral ventricle and the cisterna magna and the synthetic CSF, containing experimental compounds, is perfused from ventricle to cistern.

Another important development has been the use of brain autoradiography in the study of the distribution of radioactively labelled drugs by Roth and his co-workers. The techniques that this group has so effectively applied are discussed in a recent article (14) and many of the findings are considered further.

A third technique which has promise depends on the utilization of isolated fragments of choroid plexus. The anatomy of the plexus, especially in elasmobranchs permits the use of of micropuncture techniques, and thereby provides for the direct measurement of entry and exit of compounds into and out of the sinusoidal capillaries of the choroid plexus. This method was recently used to provide the observation that dogfish choroid plexus transports organic acid dyes (15).

Pharmacological Anatomy of the Central Nervous System

A number of new findings relate to the anatomy and the physiology of the brain, choroid plexus, and arachnoidal villi. These aid in solving old problems, but raise certain new ones. The older concept of the architecture of the brain has been drastically altered by observations with the electron microscope. Using light microscopy, the brain appeared to have a large amount of extracellular fluid. The assumption that the Na^+ and Cl^- concentrations in brain were a valid measure of ECF space[6] (about 30 to 35 per cent) was in agreement with this picture. As will be discussed below, both the light microscope picture and the 30 to 35 per cent extracellular fluid space values are misleading, and current estimates for ECF in the brain range from about 4 per cent to 15 per cent.

Electron micrographs of brain have shown consistently that the nonneuronal areas of brain are filled with glial cells. There are spaces between the cells, but these are only 150 to 200 A units wide. What appears to be extracellular space in light microscopy is the "watery" cytoplasm of the glia, particularly of the astrocytes (16 to 20). The brain capillaries as seen in the electron micrograph are without visible fenestrations and are surrounded by a typical basement membrane (17, 21). The unusual finding is that about 85 per cent of the surface of the capillaries is closely surrounded by astrocytes. The remaining 15 per cent is covered by other cellular elements (17). The apparent ECF space of brain has been estimated to be 4 per cent from electron micrographs (22).

The accuracy of the picture, so consistently seen in electron micrographs, is critical. A very small change in cell volume occurring between the normal *in vivo* situation and osmic acid fixation would alter the observed space con-

[6] A space in this sense is the volume of brain (in per cent) that the compound would occupy if it were in the same concentration as in plasma.

siderably. If the cells expanded 4 per cent before fixation, the apparent ECF space would be halved, for instance, from 8 to 4 per cent. Whole brain tissue swells after death (23, 24), but it is unclear whether this is extra- or intracellular swelling. Van Harreveld, however, has evidence that neural cells increase in size after death (25). It is important, therefore, to examine the pharmacological evidence for the extent of ECF space in brain. This is based on the use of compounds limited to the ECF space in muscle. The inulin space in the brain of nephrectomized rats is about 5 per cent, and the sulfate space is about 4 per cent in intact rats (26) and in ureter-ligated cats (27). If the same sulfate were reversibly bound to plasma protein, and recent studies suggest this may be the case (28, 29), this could be an underestimate of the true space, since ECF of brain is protein-free (see discussion that follows).

There are other reports suggesting that the 5 per cent value may be low,[7] although there is agreement that 30 to 35 per cent is too high. The electrical resistivity of the brain has been studied by Van Harreveld and he suggests that the 4 per cent figure may be low (25). Davson has measured the volume of sucrose, iodide, and inulin in brain after maintaining a constant concentration of the test drug in the subarachnoid space and plasma by repeated injection. The ECF space in spinal cord was about 12 per cent, in the brain about 5 per cent, the difference being attributed to the erratic concentrations maintained around the brain (33). Rall & Zubrod maintained a constant plasma concentration of *p*-aminohippurate or sulfanilic acid in dogfish for four days. After one or two days, the volume of these compounds in brain achieved a steady-state at about 16 per cent (34). Streicher studied the distribution of different concentrations of thiocyanate between plasma and brain in rats (35). This study should be a model for future investigations since he was careful to achieve steady-state conditions, and to correct the plasma concentrations for protein binding. With low plasma concentrations, the thiocyanate volume in brain was 4 to 5 per cent, and this rose to about 15 per cent as the plasma concentration was increased. Since CSF and brain ECF appear to be similar, it is interesting that CSF:plasma ratios of thiocyanate also increased as plasma concentrations increased, and finally approached unity at high plasma concentrations of thiocyanate (36). Thus, at the lower plasma concentrations thiocyanate may have been excluded from CSF and also from brain ECF. The higher figures for brain thiocyanate space, therefore, seem to be more likely. This would place the ECF space in brain at 8 to 12 per cent. However, all of the methods which depend on drug entry from plasma are open to a serious criticism. Since the capillaries of the CNS seem to be wrapped in cellular elements, any drug must pass through these cells to reach the ECF of brain. How can a compound, chosen to measure ECF in brain by vitue of its exclusion by cells, traverse the cellular coverings of

[7] The space occupied by a number of compounds in excised brain *in vitro* is about 15 to 25 per cent (30,31,32), but these studies are open to criticism concerning tissue viability.

brain capillaries to enter this space? Perhaps, then, Davson's *in vivo* method (33) does give the best value, since there seems to be less of a barrier at the pia-glia covering of the brain and cord (37).

Actually, it does not seem important, in understanding the pharmacology of the brain, whether this space is 4, 8, or 12 per cent. It is important, however, to realize that brain ECF is protein-free (4, 38), and, therefore, is different from the ECF of other tissues. This is probably the real significance of the classic trypan blue experiments. Since Tschirgi has shown that trypan blue is tightly bound to plasma proteins (6), it is easy to visualize that muscle tissue, with ECF-containing protein should be colored by the dye, while brain, which has no ECF protein and, into which protein does not enter (39), remains uncolored. An explanation for the slow and incomplete entry of many compounds may be found in these concepts: the brain capillaries are completely invested by a cellular wrapping, making it necessary for exchange between brain and blood to be transcellular, and beyond this cellular barrier the limited ECF space is devoid of significant amounts of protein.

Formation and Flow of Cerebrospinal Fluid

The composition of cerebrospinal fluid is shown in Table I. Glucose and urea are in lower concentration in lumbar fluid than in plasma, while ascorbic acid is higher in the CSF. The pH of CSF is 0.08 to 0.20 units lower than in blood (3). It is well established that the discrepancies between CSF and plasma ion concentrations must result from an active process. The nature of the secretory process that results in the formation of CSF is unknown. There

TABLE I

Comparison of Concentration of Compounds in Cerebrospinal Fluid and Plasma*

Compound	CSF/Plasma†
Cl^-	1.15
Na^+	1.08
Mg^{++}	1.0–1.6
K^+	0.60
Ca^{++}	0.50
HCO_3^-	0.93
Protein	0.003

* Average values for mammals, see (3, 5).

† Concentration in CSF water: ultra-filterable concentration in plasma water.

is, however, evidence implicating carbonic anhydrase. This enzyme is present in choroid plexus and glial cells (40, 41). Inhibition of carbonic anhydrase by acetazolamide causes a reduction in CSF production (42, 43, 44), and in CSF Cl^- concentration (44). A potential difference of 5 to 30 mV exists be-

tween CSF and the general ECF space of cats (45) and dogfish (46), but it is not altered by acetazolamide (46). These observations have lead Hogben and co-workers to conclude that the excess chloride in CSF (47) is the result of transport against an electrochemical gradient and that this is involved in the secretory processes of the choroid plexus (46). The role of carbonic anhydrase is visualized as providing HCO_3^- for exchange with Cl^- (4).

The mechanism of the Na^+ excess and K^- deficit in mammalian CSF may be explained by the recent observation by Bonting and co-workers that the Na^+-K^+ activated adenosinetriphosphatase exchange pump for Na^+-K^+ is present in the mammalian choroid plexus as well as in brain tissue (48). Dogfish CSF does not show the K^+ deficit, and the Na^+ excess is marginal (49). It would be of interest to know if this enzyme is present in the dogfish.

Although the mechanism by which CSF is formed remains unknown, it is now possible to measure with some precision the rate of its formation. Older methods indicate that 0.2 to 0.5 per cent of the total fluid volume was formed per min (5). A similar value has been reported for the dog, using the disappearance rate of intrathecally administered C^{14}-labeled inulin or dextran as the index of CSF flow (50). The perfusion technique of Pappenheimer & Heisey (13) has permitted a precise estimation of this parameter. They showed that inulin did not leave the perfusion fluid by diffusion or transport into brain or blood. The dilution of inulin that occurred as the fluid passed from the lateral ventricle to the cisterna magna was a function of the volume of CSF formed.[8] For the goat, Pappenheimer & Heisey obtained a value of about 200 cu mm/min as the rate of production of CSF. Using this technique in the dog, Oppelt, Patlak & Rall estimated that the rate of formation in the dog was about 0.4 to 0.6 per cent/min (51). This value is higher than, but in general agreement with, other methods [see (5)]. One important use of this technique will be in the study of the quantitative effect of inhibitors of CSF formation.

That some sort of bulk absorption or bulk flow of CSF occurs has been assumed for a long time. The prevailing anatomical picture of the arachnoidal villi, showing a solid cellular barrier between the CSF and the venous blood, prevented acceptance of the concept that CSF actually flowed, without cellular restriction, into the blood. Recent anatomical studies by Welch & Friedman have demonstrated that the structure of these villi is rather like a one-way valve (52). When the CSF pressure was greater than the venous sinus pressure, an open channel from CSF to blood could be visualized, but this was closed when venous pressure exceeded CSF pressure. They were also able to demonstrate that synthetic CSF flowed through sections of dura containing arachnoidal villi when reasonable pressures were applied on the CSF side, but no flow was demonstrable when the pressure on the blood side was

[8] If Vf = the rate of formation of CSF, Vi = perfusion inflow rate, Ci = concentration of inulin in the inflow and Co = the concentration in the outflow, then $Vf = Vi(C_i - Co)/Co$.

higher than on the CSF side. It seems clear that this is the major route of exit of CSF.[9]

Drug Exchange Between Blood, Cerebrospinal Fluid, and Brain

The purpose of this section is not to record the results of various articles in which the concentration of a drug has been measured in CSF or brain, but rather to review current data on drug distribution in the light of the possible mechanisms of exchange. This will provide, then, a framework into which specific examples of drug distribution data may be placed.

The first and simplest mechanism of exchange is passive diffusion. The characteristics of a high degree of lipoid solubility,[10] low extent of ionization,[11] and lack of plasma protein-binding virtually insure that a compound will enter brain and CSF freely and attain equilibrium rapidly (8, 9, 53). These factors are also those that favor cell entry in general and apply to such drugs as antipyrine, thiourea, thiopental, sulfanilamide, and others. These observations, well summarized in last year's review by Schanker (1), support the concept that entry into brain and CSF is dependent upon passage through intact cells, whether they be in the choroid plexus, ependyma, or glial sheath around brain capillaries. Compounds which are poorly lipoid-soluble, but ionized to a low extent and protein-bound to a slight extent, enter CSF and brain slowly, and often do not achieve a steady-state ratio of unity. Certain carbohydrates are examples of such compounds. Inulin and dextran achieved a steady-state ratio between plasma and CSF less than 0.10 (50), and inulin appeared in brain to about the same extent at steady-state (26).

The extent of ionization of a compound influences entry into CSF and brain in a number of important ways. The unionized form of a compound penetrates cell membranes much more readily than the ionized form. Therefore, increased ionization at physiological pH will decrease the fraction of unionized compound available for diffusion. This effect is also evident in its lipoid solubility when measured at pH 7.4, since the ionized moiety will, in general, remain in the polar buffer.

The effect of what Milne has called nonionic diffusion is of great importance in affecting the distribution of weak organic electrolytes (54). This refers to the effects of pH gradients on the distribution of partially ionized compounds. Weak acids, for example, are relatively excluded from areas of lower pH and concentrated in areas of higher pH. Experimental acidosis or alkalosis can therefore alter drug exchange between blood, brain, and CSF. Metabolic acidosis or alkalosis results in minimal changes in the normally

[9] For a discussion of other possible routes, see (5).

[10] This refers to the partition coefficient between some nonpolar solvent, *e.g.*, $CHCl_3$, ether, etc., and an aqueous buffer at pH 7.4.

[11] In this review, the phrase "low extent" of ionization means that less than 10 per cent of the compound is ionized at pH 7.4, a "high extent" of ionization means that more than 99 per cent of the compound is ionized at pH 7.4.

more acid CSF or intracellular pH,[12] while respiratory acidosis or alkalosis tends to alter CSF or intracellular pH parallel to plasma pH (8, 56, 57). Recent studies have focused on the effects of altered blood CO_2 tension on drug exchange. Clemedson and co-workers have shown that inhalation of concentrations of CO_2 up to 30 per cent increase trypan blue (or trypan blue-protein complex) entry into the brain of rabbits, cats, and guinea pigs (58). At the higher concentrations of CO_2, some damage to the cerebral vessels was observed. Lending and co-workers studied the effect of inhalation of 7 per cent CO_2 on the entry of radioiodinated serum albumin into CSF in puppies and adult dogs (59). In puppies, inhalation of 7 per cent CO_2 for 30 min caused a severe acidosis (plasma pH 6.86) and increased 20-fold the entry of the radioiodinated serum albumin into CSF at 30 min. In adult dogs similarly treated, the plasma pH fell only 0.05 units, and the mean cerebrospinal fluid:plasma ratios for the radioiodinated serum were not significantly different. The pCO_2 changes in all groups were comparable to the pH changes. The important observation is that young animals are much less able to maintain a normal pH and pCO_2 in the face of the CO_2 load, and, therefore, it is impossible to decide whether the blood-CSF barrier in the puppies is different from that in the adult dog.

The effects of hypercapnia (33 to 25 per cent CO_2, mean blood pH 6.81) and hypocapnia (hyperventilation at 40 respirations/min, mean blood pH 7.86) on the distribution of phenobarbital, salicylate, acetazolamide, and

TABLE II

HYPERCAPNIA OR HYPOCAPNIA ON DRUG DISTRIBUTION*

Brain or CSF:Plasma ratio × 100†

Area	Salicylate (1 hr)			Phenobarbital (½ hr)			Acetazolamide (1 hr)			Urea (1 hr)		
	↑ CO_2	N	CO_2 ↓	↑ CO_2	N	CO_2 ↓	↑ CO_2	N	CO_2 ↓	↑ CO_2	N	CO_2 ↓
Cerebral cortex	44	17	11	120	97	72	20	6	5	40	29	16
Cerebral white‡	38	8	4	108	59	40	10/3	2/1	5/1	12	10	5
Caudate nucleus‡	44	13	9	109	80	53	13/6	14/5	8/2	19	21	8
Hypothalamus	47	15	9	106	82	49	11	8	8	22	18	11
CSF	48	18	12	48	39	42	14	8	7	26	26	13

* Data from Goldberg *et al.* (60). Normal values: phenobarbital (63), acetazolamide (61), urea (62). These latter two were converted from dry wt to wet wt by values from (61). Blood urea concentrations were converted to plasma by factors in (64), assuming a hematocrit of 45 per cent.

† Calculated on a wet weight basis for brain, and per ml basis for CSF and plasma. ↑ CO_2 and ↓ CO_2 refer to hyper- and hypocapnia, N to normal.

‡ For acetazolamide the values for the ventricular surface precede, and those for the centrum follow the ratio symbol.

[12] This is caused by the slow exchange of bicarbonate between blood, CSF, and brain (5, 55).

urea in the brain of the cat have been studied by Goldberg, Barlow & Roth (60). Blood, CSF, and neuroanatomical areas of the brain were sampled after ½ or 1 hr and gross radioautographs of brain sections were obtained. Control data were presented only for salicylate and therefore comparison was rendered difficult. The controls presented in Table II are derived from previous publications (61, 62, 63). Salicylate and phenobarbital showed consistently higher brain:plasma ratios after hypercapnia and lower ratios after hypocapnia. The greater difference occurred with salicylate, which has the lower pK. It was suggested that hypercapnia similarly increased, and hypocapnia lowered the brain:plasma ratio for acetazolamide and urea. Inspection of Table II supports this only partially. Hypercapnia increased brain entry of acetazolamide but did not effect urea. Conversely, hypocapnia decreased urea penetration, and did not affect acetazolamide entry into brain. Acetazolamide, a weak acid like phenobarbital, might be expected to show increased entry during hypercapnia. Plasma protein binding can be influenced by pH, but this was not determined in these experiments, and the effect of pH on acetazolamide binding to plasma proteins is unknown. Since these data were not obtained at a steady-state, but at a time when the relative concentrations were changing rapidly, interpretation is rendered difficult. The situation with urea is also unclear, as these data also are from an unsteady-state situation. Since urea is virtually nonionized at these pH values, no effect related to pH would be expected. Therefore, the failure of hypercapnia to increase entry is reasonable; no ready explanation is available for the decreased entry in hypocapnia. Certainly, the CO_2 concentration may have some specific action on the barrier, although this seams unlikely since the results reported in this study with salicylate and phenobarbital are consistent with nonionic diffusion, which requires a virtually intact lipoid membrane (54). Further, an earlier report showed no effect of hypo- and hypercapnia on the distribution of sulfanilamide or antipyrine between CSF and plasma (8). Another problem with the conduct of the study with urea and the other drugs is that tissue concentrations were determined on a dry weight basis. To convert to a wet weight ratio between brain and plasma, separately determined dry weight to wet weight factors were used. These factors were obtained from normal cats. It is possible that fluid shifts might occur in severely acidotic, hypercapnic, or alkalotic hypocapnic animals and thus introduce a systematic error.

As mentioned before, reversible binding of a drug to plasma proteins can strongly affect its distribution. Anton has demonstrated this recently in an interesting paper (7). Sulfinpyrazone, itself extensively bound to plasma protein, can displace other drugs from binding sites. Using extensively bound sulfonamides as the test drugs, Anton has shown that displacement from plasma proteins by sulfinpyrazone decreased the plasma concentration by allowing more sulfonamide to enter the tissues and increased tissue (including brain) to plasma ratios. Neither of these effects was seen when a sulfonamide was used which was only slightly bound to plasma protein.

One of the most interesting compounds which fails to achieve diffusion

equilibrium between CSF and plasma is acetazolamide. It is highly bound to human and canine plasma protein (44), is a weak organic acid, pK's of 7.4 and 9.1, and is not unusually lipoid-insoluble (65, 66). In both man and dog, steady-state CSF:plasma ratios are significantly lower than would be predicted by binding alone (44, 67). In the cat, low CSF:plasma ratios were observed, but no protein binding studies were performed (61). Interestingly enough in the dogfish, the CSF:plasma ratio for acetazolamide approaches unity (40). An albumin component is lacking in dogfish plasma, and this drug is not bound to the plasma protein of this species. Therefore, protein binding seems to be partially responsible for the exclusion of acetazolamide from mammalian CSF. As other mechanisms must also be involved, it would be important to study this drug in the manner in which Pappenheimer and his co-workers studied the active transport of other compounds. In addition, the relatively rapid turnover rate of CSF (see following discussion) compared to the slow penetration of the drug, may prevent the attainment of a CSF:plasma ratio close to unity.

Besides nonspecific and reversible binding to plasma proteins, it appears that compounds may be bound to specific intracellular components. Barbiturates are bound to some components of brain (68). Acridine antimalarials appear to be bound to intracellular components, perhaps nucleic acids (69). For many drugs, the affinity is localized to certain neuroanatomical areas within the brain. Bilirubin localizes in the basal ganglia in kernicterus. Roth, Schoolar & Barlow, in an extensive series of studies, have explored the neuroanatomical localization of a number of important pharmacological agents. Using autoradiographic techniques, they have shown the localization of isonicotinic acid hydrazide in the hippocampus (70) and of acetazolamide in the hippocampus, caudate nucleus, and hypothalamus (61). They have shown that many drugs are relatively excluded from white matter in comparison with grey matter. This important difference is related to the myelin sheaths in the white matter which apparently act as diffusion barriers (14). In young animals this general pattern is not seen and this observation can be correlated with the lack of myelinization in the developing brain (63).

In addition to binding to cellular proteins in the brain, binding to the cellular components of blood must be considered. A recent study of the distribution of quinine in the dogfish by Rall & Zubrod (71) revealed that this compound was so highly concentrated in or on white and red blood cells that it was impossible to arrive at the true plasma concentration. Damage to as little as 1 per cent of the red or white cells in processing the blood for analysis released enough quinine to increase the plasma concentration three-fold, and spuriously low CSF:plasma ratios were found. True CSF:plasma concentration ratios could be obtained only by indirect means.

An important factor influencing exchange of drugs between blood and CSF is the rapid bulk turnover of CSF (13). Recent data obtained by the perfusion method indicate a turnover rate of about 0.5 per cent per min or 30 per cent per hour (51). The implication of this rapid flow of CSF is that a

compound which does not enter it at a fairly rapid rate will equilibrate in CSF at some concentration less than that found in the plasma. This may be one reason why the CSF®plasma ratio for acetazolamide is relatively low in mammals.[13]

The rapid turnover of CSF also may be the reason why inulin is effectively excluded from it. The steady-state-CSF:plasma ratio is about 0.07 in nephrectomized dogs. The absolute entry rate of inulin, a large and lipoid insoluble molecule, might be expected to be low compared to the turnover rate of CSF. If, however, the CSF were stagnant in the sense that there were no bulk flow but only exchange of water and solute through a cellular membrane, the concentration of inulin should slowly increase with time and approach that of plasma if the plasma concentration remained constant. In experiments lasting as long as 4 or 5 days, a steady-state ratio of 0.07 between CSF and plasma inulin was reached at about 2 days (50). Since inulin is inert, not protein-bound, and not likely to be actively transported, the existance of bulk removal of CSF was offered as the explanation for these data.

Recently, a typical example of an active transport process has been shown to occur *within* the CNS (12). Pappenheimer, Heisey & Jordan demonstrated that phenol red and diodrast were removed at a much faster rate than was creatinine or inulin from artificial CSF perfusing the cerebral ventricular system of goats. This removal of diodrast from the perfusion fluid occurred when the diodrast concentration in the blood was greater than that in the perfusion fluid. Addition of *p*-aminohippurate or phenol red to the perfusion fluid inhibited the removal of diodrast. When the concentration of diodrast or phenol red was progressively increased in the perfusion fluid, the removal of these organic acids was relatively decreased. In the goat, the Tm, or transport maximum, was about 3μg/min for these drugs. These observations clearly define a stereospecific active transport mechanism, capable of removing organic acids from the CSF, but they do not localize this process. A stop flow analysis suggested that the region of the active absorption was the fourth ventricle and the cisterna magna. Since Cameron (73) and Lumsden (74) showed that embryonal choroid plexus grown in tissue culture transported phenol red, Pappenheimer and co-workers suggested that the choroid plexus of the fourth ventricle was the site of the active absorption. Prockop, Schanker & Brodie (75) have shown recently that active absorption of organic acids from the CSF also occurred in the rabbit. Rall & Sheldon have shown that this active absorption occurred *in vitro* in fragments of isolated dogfish choroid plexus (15). When chlorphenol red in a balanced salt solution bathed these fragments of choroid plexus the dye concentrated in the capillary lumen, while the choroidal epithelial cells remained uncolored. In this isolated system competition between other organic acids and chlorphenol red

[13] Preliminary data indicate that the turnover rate of dogfish CSF is much lower than that of mammals (72). This might explain in part why acetazolamide approaches equilibrium in this elasmobranch.

was demonstrated. Cold, and low concentrations of 2,4-dinitrophenol, inhibited chlorphenol red uptake, indicating dependence upon tissue metabolism. These observations in the goat, rabbit, and dogfish offer persuasive evidence that active absorption of organic acids occurs as a mechanism by which these compounds leave the CSF rapidly.

The similarity between the choroid plexus cells and the proximal renal tubular cells is immediately apparent. Electron microscopy of the choroid plexus and the proximal renal tubule reveals a number of common features such as the brush border, oriented mitochondria, the presence of microbodies, and the interdigitated cell borders (76, 77). It is tempting to speculate, therefore, that a process for transporting organic bases also exists in the choroid plexus, as it does in the proximal renal tubule (78). Further, perhaps the choroidal epithelial cells are as nonselective toward the organic acids that are transported as are the proximal tubule cells (79).

It was mentioned that the active absorption system of the choroid plexus has a low transport maximum, amounting to about 3μg/min of phenol red or diodrast. This further emphasized the importance of protein binding in the distribution of a drug between blood and CSF. With a minimal protein binding, the amount of drug entering may be high enough to saturate the absorption mechanism, and result in a CSF:plasma ratio approaching unity; with extensive protein binding, much less free drug is available to enter the CSF, and the active absorption may virtually be able to clear the CSF of this compound.

An interesting question is raised by the competitive inhibition of this transport mechanism by other organic acids. Can a high plasma concentration of one acid influence the steady-state entry of a second acid? No data are yet available, but this could be of considerable therapeutic and toxicological importance.

A number of recent articles have suggested that an active transport system for bromphenol blue exists at the empendymal junction of the CSF and brain. Perfusion from one lateral ventricle to either the aqueduct of Sylvius or the cisterna magna with bromphenol blue, resulted in a blue coloring of the adjoining brain tissue, more pronounced in the grey than the white matter (11, 80). When this perfusion was begun immediately after the death of the animal, much less blue staining was noted. This observation was interpreted as suggesting the active transport of bromphenol blue into the brain. It seems quite premature to consider this active transport[14]. Bromphenol blue binds extensively to plasma proteins[15], and presumably also to the proteins in the cells of the CNS. Therefore, binding to and coloring of the brain tissue might be explained by diffusion from the protein-free CSF and progressive binding in the brain. That this process stops or decreases at death

[14] In the dogfish choroid plexus, bromphenol blue does not seem to be actively transported; it stains both cells and lumen, and is unaffected by cold (15).

[15] It is used in paper electrophoresis of plasma proteins to stain the protein.

might be explained by the recent observation that cerebral tissue pH falls very rapidly after death (81). Therefore, bromphenol blue, an organic acid, would be excluded as the pH of the brain tissue falls.

Histamine (82) and acetazolamide (61) similarly appear to pass into brain tissue from the CSF under certain circumstances. It is impossible, from the data given, to evaluate these observations in terms of active mechanisms.

Epinephrine also passes into the brain and is absorbed into the general circulation when injected into the CSF (83). This observation becomes increasingly interesting in the light of observations made by Titus and coworkers that slices of cerebral cortex, when incubated in a medium containing the related catecholamine, norepinephrine, take up this compound by a process that has many characteristics of active transport (84). This uptake is inhibited by ouabain and reserpine (85). These preliminary observations have implications concerning catecholamine distribution in the body.

Uptake and exclusion of metabolites by the brain seem, on theoretical grounds, to require intervention of active processes. Many studies of the exchange of glucose, amino acids, and other substances are currently in progress or have been reported recently. The same principles applied to the study of drug exchange should govern the investigation of these important compounds. This aspect of the problem will be left for future reviewers.

Clinical Implications of Blood-Brain Barrier Studies

The principles discussed above are clearly relevant to the use of drugs in patients and to an understanding of the natural history of disease insofar as these depend upon the exchange of endogenous and exogenous compounds between blood, brain, and CSF. Since insufficient clinical data are available for systematic review, the authors have selected a few reports in clinical and experimental situations to indicate some aspects of "barrier" physiology important to the physician.

Drug transfer into the CSF of patients is often studied with inadequate attention to the experimental design mentioned previously: constant plasma concentration, repetitive sampling of CSF, determinations of difference in pH between CSF and blood and the amount of drug in plasma water. Only when these conditions are met can the extent and the rate of drug (or metabolite) transfer be defined quantitatively. The methods developed for the dog (8) have been adapted for patients (86), sampling lumbar rather than cisternal fluid. Steady-state ratios and transfer rates were established for antipyrine, sulfadiazine, and *p*-aminohippurate. The major difference from the dog was the slow rate in man of achieving a steady-state concentration ratio between blood and lumbar fluid.

The effects of physiological abnormality upon distribution of drugs or metabolites between blood and CSF or brain have not received attention equivalent to their potential importance. In anesthetized patients, the possibility of altered blood gases, acid-base balance, and blood flow frequently exists. Most anesthetic agents are lipoid-soluble drugs which, under normal

conditions, completely and rapidly exchange between blood, CSF, and brain. Hence, anoxia, hypotension, or metabolic or respiratory acidosis will not alter the rate or degree of drug exchange. The question might be raised as to what happens to the distribution of other drugs such as morphine, salicylate, atropine, and digitalis, which may also be in the circulating blood.

The net increase or decrease of drug concentration in the CNS may be difficult to predict as may be seen from a consideration of pH gradient changes. For example, when pH is higher in CSF than in blood, weakly acidic drugs such as salicylate reach a higher concentration in CSF than in blood. At the same time, similar pH gradients between blood and urine may increase the renal clearance of salicylate and lower the plasma concentrations (87). In addition, salicylate distribution between blood and other tissues will be altered. The net change of salicylate in the CNS cannot be predicted, and actual measurements should be made in man before recommending that salicylate toxicity be treated by creating a metabolic acidosis. Acetazolamide had a deleterious effect on the course of salicylate toxicity (88). With weakly basic materials such as ammonia, the reverse would occur, i.e., metabolic alkalosis would increase the ammonia in the CNS (56).

Similarly, the effects of other altered physiologic states upon the exchange of drugs between blood, CSF, and brain have not been assessed in man though the foregoing observations suggest the need for such study. In hypoglycemia in rats, there occurred a rise in aspartic acid and ethanolamine in brain and a fall in glutamic acid, glutamine, alanine, γ-aminobutyric acid, and glycine (89). Hypoxia or hypercapnia in puppies (but not in adult dogs) increased the CSF:plasma ratio of glutamine oxalacetic acid transaminase and I^{131}-albumin 5 to 20-fold and of lactic dehydrogenase threefold (59, 90). Hypotension in dogs maintained for 4 hr did not alter the entry of sulfanilic acid, sulfanilamide, or antipyrine into CSF (91). Repeated electroshocks to rabbits were associated with increased amounts of I^{131}-albumin in brain (92). Air embolization through the carotid arteries of rabbits and cats, transiently permitted an increased passage into brain of I^{131}-albumin (93).

It has always been assumed that infection in the CNS, especially in the meninges, increases the permeability of the barriers to drugs (94). Streptomycin transfer increased during active tuberculous meningitis (95), as did the transfer of bromide (96)[16]. Both returned to normal as the meningitis regressed. No changes in bromide transfer were noted in other types of lymphocytic meningitis (96). Similar qualitative observations have been made for penicillin (98), and oxytetracycline (99).

The behavior of glucose in CSF during meningitis presents some paradoxes (100). Normally, the CSF:serum ratio for true glucose is about 0.8 within a wide range of serum concentrations (101). Viral meningitis causes

[16] It should be noted, however, that with bromide, as in the case of thiocyanate, the CSF:plasma ratio increases proportionately to the plasma bromide concentration (97).

no change in CSF glucose; bacterial meningitis is associated with a moderate fall, and tuberculous meningitis with a marked fall (100). With increased permeability one would assume that CSF glucose would approach serum glucose[17], but this is not the case. Increased metabolism of glucose by leucocytes or bacteria has been invoked to explain the fall in CSF glucose in the presence of increased permeability.

Some of these problems have been resolved by Petersdorf & Harter (100). Using pneumoccoccal meningitis in irradiated and normal dogs, they found that bacteria alone or granulocytes alone did not explain the decrease. Both together, acting synergistically, accounted for the fall in glucose. They concluded that in the presence of bacteria there is increased phagocytosis with a resultant sharp rise in glucose consumption. However, the marked fall in CSF glucose in tuberculous meningitis where there are relatively few bacteria and leucocytes, is not fully understood. While no similar situations are known for drugs, these observations emphasize the difficulty of studying drug exchange in the presence of metabolism of the drug (or metabolite) within the CNS.

The availability of several drugs which induce regression of acute leukemia has permitted leukemic infiltration of the meninges to become a well-recognized phenomenon (103). Cortisone and its derivatives readily cause regression of cortisone-sensitive meningeal leukemia (104), although no data are available on cortisone entry into CSF or brain. The folic acid antagonist, methotrexate which is ionized to a high extent, fails to achieve significant concentrations in the CSF after systemic administration (105). Signs of meningeal leukemia may appear even though the patient otherwise is in complete remission. It is possible that the meningeal focus could be responsible for relapse. Intrathecal methotrexate is about as effective as brain x-irradiation in bringing about regression of advanced meningeal leukemia (104, 105).

6-Mercaptopurine which is ionized to a low extent, readily enters CSF when given to dogs by continuous intravenous infusion (106). It, too, is ineffective in preventing meningeal leukemia when given by mouth. It is rapidly metabolized by the liver to thiouric acid and other inactive compounds (107). Thus, under usual clinical conditions, the blood concentration of the active component, 6-mercaptopurine, is not maintained for a long enough period to allow therapeutic amounts to reach the CNS. Mercaptopurine is apparently too toxic to administer intrathecally (104).

There is increasing recognition of the importance of pharmacological disposition of drugs in the newborn and premature infant. Several drugs well tolerated by older children are quite toxic for young infants (108), and the blood-brain barrier may play a role in some of these situations. One of the most interesting general problems relates to the slow appearance after birth of several enzymes related to metabolic transformation of drugs and other compounds of biologic importance. Chloramphenicol, for example, in newborns and premature infants, produced vascular collapse and death in asso-

[17] Blood and CSF glucose are equal in the premature nonmeningitic infant (102).

ciation with unexpectedly high blood concentrations (109). It was found that the infants had a high ratio of free to glucuronide-conjugated drug in the blood, and that they excreted relatively small amounts in the urine (110). In guinea pigs, the enzyme glucuronyl transferase was lacking in fetal liver and low in newborns (111). It is assumed that this deficiency is responsible for the human infants' inability to form the glucuronide. The relation to exchange of drugs between blood, brain, and CSF is twofold: (*a*) because of the slower renal clearance of free drug, higher plasma concentrations are presented to the CNS barriers; (*b*) the unaltered drug has a higher pKa and is generally more lipoid-soluble than the glucuronide and would, in general, reach a higher CNS concentration. Other substances which undergo glucuronide formation are morphine, thyroxine, tetrahydrocortisone, and bilirubin (108).

While the role of pH gradient in redistribution of drugs or metabolites has not been studied in premature infants, it should be noted that they are highly susceptible to induced metabolic acidosis, an ideal situation for the development of a relatively alkaline CSF. In the premature, there is a relative inability to excrete acids (112). This, per se, means higher plasma and CSF concentrations of weakly acid drugs such as salicylate and sulfonamides. In addition, small amounts of ingested acids such as lactic acid will induce a severe metabolic acidosis (113). Weakly acid drugs or metabolites would then tend to reach higher concentrations in CSF than in the plasma water.

The deposition of bilirubin in basal ganglia of the brain in jaundiced newborn infants is of some interest in relation to exchange of compounds of biologic interest between the blood and CNS. Bilirubin is very extensively bound to serum albumin and no free bilirubin appears in ultrafiltrates of plasma containing bilirubin until the concentrations of bilirubin are above 80 mg per cent (114). Nevertheless, kernicterus develops with plasma bilirubin concentrations of less than 20 mg per cent (115). One possible explanation is that the barriers are more permeable in infants. In newborns, fluorescine, after intravenous administration, readily appears in CSF, and CSF albumin is moderately elevated (116). In prematures, where the danger of kernicterus is greater, CSF albumin is even higher and the CSF : plasma ratio for glucose is unity (102). The alteration in permeability may be related to birth trauma but this is not necessarily the only possible mechanism, as experimental studies suggest that newborn animals have incompletely developed blood-brain barriers (117). However, other studies indicate normal barriers (63, 118). It should be pointed out that the albumin-bound bilirubin thus introduced into CSF, would continue to be extensively bound and probably not available to enter brain cells (119). Since kernicterus occurs at CSF bilirubin concentrations of 2 mg per cent and below (120), some mechanism of dissociation of bilirubin must be present to explain the accessibility of bilirubin to brain cells.

There is some correlation between the degree of indirect bilirubinemia and the likelihood of kernicterus (121). Indirect bilirubin is the lipoid-soluble free

pigment (122). Direct bilirubin, the water-soluble diglucuronide (with small amounts of monoglucuronide), is formed through the action of glucuronyl transferase (123). The unconjugated bilirubin is poorly excreted by kidney and liver, and deficiency of the enzyme results in high indirect bilirubin concentrations in blood and CSF (123). Both the unconjugated bilirubin and the diglucuronide are almost completely bound to albumin (124), and hence cannot pass from blood to CSF. If abnormally present in CSF they are not free to diffuse into nerve cells (115). If albumin-bound unconjugated bilirubin in CSF undergoes slight dissociation, the free bilirubin, being lipoid-soluble, would enter nerve cells. If the nerve cell-free bilirubin complex has a dissociation constant smaller than albumin-bilirubin, then equilibrium would be shifted toward the cell with resultant cell cumulation.

There is another recently described circumstance which is clearly responsible for some instances of kernicterus and may provide a clue to the common pathogenetic mechanisms. A number of exogenous and endogenous anions can displace bilirubin from albumin (115, 119). Thus, kernicterus with high mortality was shown to be associated with the use of sulfisoxazole in premature infants (125). Salicylates, several other sulfonamides, caffeine sodium benzoate, and hematin have been shown to displace bilirubin (119). Whether the anion in blood or CSF is responsible for the dissociation is unknown, but further investigation is needed of the role of anions in kernicterus. It should be recalled that during metabolic acidosis anions of weak acids may accumulate in the CSF and brain. One should also consider (*a*) the role of anions transferred to the fetus during hyperbilirubinemia, and (*b*) anions within basal ganglia, as possible reasons for the specific localization of bilirubin. Since hematin can displace bilirubin from albumin and since this pigment arises during acute hemolysis, it is possible that the hemolytic episode provides both the excess bilirubin and the anions which free the bilirubin from the albumin.

In summary then, kernicterus probably results from the simultaneous occurrence of hyperbilirubinemia, glucuronyl transferase deficiency, increased permeability of the newborn blood-brain barrier, and presence of a displacing anion.

Concluding Remarks

Drugs can exchange between the brain or CSF by either diffusion or some form of active transport. Exit of drugs from the CSF is aided by a third mechanism, bulk flow. The "barriers" separating blood from brain or CSF are cellular in nature. The choroid plexus presents a solid cellular layer as a barrier between blood and CSF. The capillary wall, completely surrounded by glia and other cellular elements, presents a cellular barrier between blood and brain. The extracellular fluid in brain is limited in amount, and is essentially protein-free, more or less resembling CSF.

The emphasis in this review has been placed on passive diffusion of compounds between blood, brain, and CSF. It is clear that active transport

mechanisms should not be invoked until passive diffusion has been shown to be unable to account for the experimental observations. There are many factors which affect the exchange of drugs and metabolites into and out of the central nervous system. It is clear that the exchange of compounds between blood, brain, and CSF cannot be divorced from the disposition and modification of the drug elsewhere in the body or from the effects of systemic physiology and biochemistry upon the cellular membranes separating these areas. *In vivo*, this means that it is difficult by *a priori* reasoning to predict the effect of altered experimental conditions or disease on the amount of active agent reaching various regions of the CNS. There is no substitute for quantitative study of the mechanism of exchange under the actual experimental or clinical circumstance.

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