THE IODIDE SPACE IN RABBIT BRAIN

Thesis by

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To my husband and my daughter

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ABSTRACT

In the present investigation labeled iodide was used to investigate the interrelationship between brain, blood and cerebrospinal fluid, to examine active transport across the blood-brain- and the blood-cerebrospinal fluid barriers, and to estimate the extracellular space of the brain.

The iodide space in the brain and the iodide concentration in cerebrospinal fluid after intravenous administration of radioactive iodide are determined by the following mechanisms. Iodide passes into the cerebrospinal fluid but active transport in the choroid plexus moves most of the iodide back again into the plasma, keeping the concentration at a very low value. An extracellular fluid is formed at the blood-brain barrier possibly in a similar way. The iodide concentration of this fluid is unknown but is probably higher than that in the cerebrospinal fluid. Diffusion of iodide across the brain-cerebrospinal fluid barrier transports this ion from the brain into the cerebrospinal fluid which is constantly renewed "sink action".

The iodide space was found to be 2.4% four to five hours after the intravenous administration of ^{131}I, the iodide content of the cerebrospinal fluid was 1.2% of that of the TCA serum filtrate. The iodide space increased to 10.6% in preparations in which in addition to ^{131}I unlabeled iodide (to a serum concentration of 25 to 50 mM) was administered to saturate the active transport processes in the choroid plexuses and blood-brain barrier. The iodide activity of the cerebrospinal fluid

in these experiments increased to 29.3% of that in the TCA serum filtrate. In experiments in which the inhibitor of iodide transport, perchlorate (8 mM), was injected intravenously with the $^{131}I^-$, the iodide space was 8.2% and the iodide concentration in the cerebrospinal fluid 26.4%. These experiments demonstrate the effect of saturation and inhibition of active transport on the iodide space. They show furthermore that the depression of the active transport did not raise the iodide concentration in the cerebrospinal fluid to the plasma concentration. The relatively low (1/3 of that in the serum TCA filtrate) iodide concentration in the cerebrospinal fluid under these circumstances was ascribed to a differential permeability of the blood-cerebrospinal fluid barrier for iodide and chloride.

The sink action can be eliminated by perfusion of the ventricles with an artificial cerebrospinal fluid containing iodide. Ventriculocisternal perfusion with $^{131}I^-$ alone resulted in an iodide space of 7.2% after 4.5 hours. An iodide space of 10.2% was determined by a combined intravenous administration and ventricular perfusion with an artificial cerebrospinal fluid containing the same concentration of ^{131}I as present in the plasma. When in similar experiments perchlorate was administered intravenously, the iodide space rose to 16.8%. The iodide space determined by simultaneous intravenous injection and ventricular perfusion with both labeled and unlabeled iodide, in a concentration sufficient to saturate the active transport, was 20.8%. In the latter instances the sink action is eliminated and also active transport is inhibited or saturated. It was postulated that under

these conditions the iodide concentration in plasma and brain extracellular fluid are approximately the same. The use of the iodide space as a measure of the brain extracellular space was discussed.

INTRODUCTION

Determination of extracellular space.

In most tissues the extracellular space can be determined by the intravenous administration of a compound which does not readily penetrate the cell membrane but passes quickly through the capillary wall. After allowing time for equilibration of the compound in plasma and extracellular material, the extracellular space is estimated by determining the concentrations of the compound in both serum and tissue. The most common compounds used for this purpose are inulin, sucrose, thiocyanate and radioactive tracers such as thiocyanate and iodide. The extracellular space is computed in the following way, assuming that the electrolyte composition in the extracellular fluid and the plasma are the same and that the marker does not penetrate into the intracellular compartment.

$$\text{Extracellular space} = \frac{\text{concentration of the compound in tissue}}{\text{concentration of the compound in plasma}} \times 100$$

A ^{24}Na space of 11% was found in this way in rabbit muscle (Hahn, Hevesy and Rebbe, 1939). With inulin an extracellular space of 9.6% was estimated in rat muscle by Creese, d'Silva and Hashish (1955), of 10.0% by Law and Phelps (1966). The naturally present chloride and sodium have in the past been used to determine the extracellular space on the (erroneous) assumption that these ions are present exclusively in the extracellular compartment. Since a certain percentage of these ions are present in the cellular elements such values are too high.

Wilde (1945) determined a chloride space of 17% for rat muscles in this way. The sulfate space determined with labeled SO_4 was found to be 60 to 80% of the chloride space (Walser, Seldin and Grollman, 1954). The inulin space was 80% of the chloride space after 1 to 2 hours of equilibration (Cotlove, 1954).

The application of these methods to the central nervous system is difficult and uncertain because of the presence of a barrier between blood and brain (the blood-brain barrier) which prevents the quick penetration of the marker compounds into the nervous tissue. Goldmann (1909) showed that the intravenous infusion of the dye, trypan blue, did not result in staining of the central nervous tissue. However, when this dye was introduced directly into the ventricles of the brain (i.e., into the cerebrospinal fluid system), the tissue was easily stained. Later it was found that many other compounds are not readily transported from the blood into the brain tissue. This supported the idea of the presence of a barrier between blood and brain. The blood-brain barrier isolates the brain and prevents rapid changes in the surrounding of the nerve cells. Although penetration of many compounds through the blood-brain barrier is slow, it occurs at a measurable rate. It was believed, therefore, that if sufficient time is allowed, an equality of marker compound concentrations in plasma and extracellular material of the brain could be achieved, making it possible to estimate the extracellular space in brain tissue.

Based on this concept Woodbury (1958) determined a SO_4 space of 3.9% in rat brain by using labeled sulfate. After 4 hours, this

value was reached, which remained at the same level for 16 hours but then increased. He also found an inulin space of about 4 to 5% after 16 hours equilibration. Barlow, Domek, Goldberg and Roth (1961) determined a $^{35}SO_4$ space between 2 to 5.5% in cats after maintaining a steady level of labeled sulfate in the plasma for 4 to 8 hours. Morrison (1959) found an inulin space of less than 5% in dogs and rats after 3 hours. Reed and Woodbury (1963) estimated a 6% iodide space in rats 4 hours after intravenous injection. Streicher (1961) determined a thiocyanate space of 4 to 17% in rat brain after 1/2 to 2 hours. The larger values were roughly correlated with a higher thiocyanate concentration in the plasma. The results of these investigations, in which the marker compounds were administered intravenously led to the acceptance of a small (3 to 5%) extracellular space in central nervous tissue.

Composition of cerebrospinal fluid.

The cerebrospinal fluid was at one time considered as a plasma ultrafiltrate (Holmes and Tower, 1955). The solvents in plasma and cerebrospinal fluid would be distributed in accordance with a Gibbs-Donnan equilibrium.

The physical requirements of a typical Gibbs-Donnan system are, first, that a membrane, separating two electrolyte solutions, is permeable for all ion species but one, and secondly, that all water movements across the membrane are prevented by a hydrostatic pressure difference between the compartments. Such a system can be schematized

as in Fig. 1A in which it is assumed that a membrane separates the two compartments I and II, and that the diffusable ions are Na^+ and Cl^-. The indiffusable ion is indicated by An^-. It is assumed that the two compartments are of equal and constant volume and that at the start the Na^+ concentration is the same in both compartments, and is equal to the Cl^- concentration in II and the An^- concentration in I. The osmoconcentrations are thus the same in the two compartments. In Fig. 1B the concentrations of these ions are indicated by the heights of the columns in the initial state.

Chloride ions will now start to diffuse across the membrane from II to I, and since equality of the positive and negative charges has to be maintained in each compartment, Na^+ ions have to accompany the Cl^- ions. Thus the Na^+ concentration becomes higher in I than in II. Then Na^+, accompanied by Cl^-, starts to diffuse back from compartment I into II. After some time the diffusion of NaCl from I into II equals that from II into I, and a Donnan equilibrium has been reached. The concentrations of the ions under these conditions are shown in Fig. 1C. In compartment I, the Na^+ concentration has increased, the An^- concentration is unchanged, and it now contains Cl^-. In compartment II, the Na^+ and Cl^- concentrations are equal but decreased. The Gibbs-Donnan equilibrium demands that the products of the concentrations of the diffusable positive and negative ions, on the two sides of the membrane, are equal, i.e.

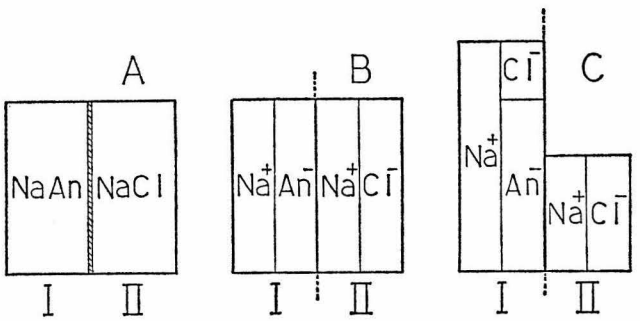

Fig. 1. A shows two electrolyte solutions NaAn and NaCl in compartments of equal, not expandable volume (I and II), separated by a membrane permeable for Na^+ and Cl^- but not for An^-. B and C show the initial and equilibrium concentrations of the ions respectively, indicated by the heights of the columns.

$$[Na^+]_I \times [Cl^-]_I = [Na^+]_{II} \times [Cl^-]_{II}$$

or

$$\frac{[Na^+]_I}{[Na^+]_{II}} = \frac{[Cl^-]_{II}}{[Cl^-]_I} \qquad (1)$$

for a divalent cation such as Ca^{++} the Gibbs-Donnan equilibrium demands the following:

$$[Ca^{++}]_I \times [Cl^-]_I^2 = [Ca^{++}]_{II} \times [Cl^-]_{II}^2$$

or

$$\frac{[Ca^{++}]_I}{[Ca^{++}]_{II}} = \frac{[Cl^-]_{II}^2}{[Cl^-]_I^2} \qquad (2)$$

If the original ion concentrations in the example shown in Fig. 1 are designated as c, and the concentration of the Na^+ and Cl^- ions which passed from compartment II into I as x, then at equilibrium, in compartment I

$$[Na^+]_I = c + x \qquad [Cl^-]_I = x$$

and in compartment II,

$$[Na^+]_{II} = c - x \qquad [Cl^-]_{II} = c - x$$

by substituting equation (1)

$$x\,(c + x) = (c - x)^2$$

or

$$x = 1/3\ c$$

The osmoconcentration in compartment I has become greater than that in II. The difference can be written as

$$(2\ c + 2\ x) - (2\ c - 2\ x) = 4\ x$$

The difference in osmoconcentration in this special case will be $4/3$ C. This represents a difference in osmotic pressure of RT $4/3$ C (atmospheres) in which R is the gas constant and T the absolute temperature. Assuming that we started with salt concentrations of 1 mole in the two compartments, then the osmotic pressure, at equilibrium and at 25°C, would be 32.6 atm. higher in compartment I than in compartment II. A Donnan equilibrium can be established only when the walls of the compartments are strong enough to overcome the osmotic pressure created in such a system. If the walls lack mechanical strength then the difference in osmotic pressure between the compartments results in a water movement from the compartment with lower concentration (II) to that with the higher concentration (I). If water passes from II into I, the concentrations of the Na^+ and Cl^- ions will increase in II, and the ion concentrations in I will decrease. Then a redistribution of the Na^+ and Cl^- ions will take place, according to the Donnan rule, resulting

in an additional transport of NaCl from II into I increasing the osmoconcentration in the latter compartment. This movement of electrolytes and water will continue until all the NaCl solution of compartment II is transported into compartment I.

In a Donnan system at equilibrium, a potential difference develops across the membrane. Diffusion of Na^+ and Cl^- tends to equalize the concentrations of the ions in both compartments, which will result in a small increase of the negative ions in compartment I and an excess of positive charges in compartment II. The resulting potential difference will counteract the movement of more Cl^- to compartment I and of Na^+ to compartment II. In other words, at equilibrium, there is equality between the forces of diffusion which tend to move ions from the compartment with higher concentration to that with the lower concentration, and the potential which tends to move the ions in the opposite direction. The potential in volts is given by the Nernst equation:

$$E = \frac{RT}{zF} \ln \frac{[Na^+]_{II}}{[Na^+]_I} = \frac{RT}{zF} \ln \frac{[Cl^-]_I}{[Cl^-]_{II}}$$

in which z represents the valency of the ion and F is Faraday's constant.

From the nature of the Donnan equilibrium, which results in differences in osmotic pressures between compartments, balanced by considerable hydrostatic pressures, it can be predicted that such a system is rare in biological systems. An exception is the system formed

by blood-plasma and tissue fluids separated by the capillary membrane, in which the blood pressure can provide the necessary hydrostatic pressure. Since cell membranes generally lack mechanical strength, no true Donnan equilibrium can be expected between cells and their surrounding. The differences in osmotic pressure which develop even at slight differences in concentration of the extra- and intracellular compartments of indiffusable compounds cannot be balanced by sufficient hydrostatic pressures. An equilibrium can exist in such systems only if additional permeability restrictions of the membrane are present, as in the case of nerve and muscle, where the membrane is considered to be impermeable for large intracellular organic anions and effectively impermeable for Na^+. Such a system is stable since the effective impermeability of the membrane for Na^+ prevents the transport of NaCl from the outside into the cells as would occur according to the Donnan rule.

Since the cerebrospinal fluid is believed to be formed at least in part by the choroid plexuses the conditions for a Donnan equilibrium are present since the capillary pressure could balance a difference in osmotic pressure between filtrate and plasma. Davson (1956) and Pollay (1966) compared the composition of an ultrafiltrate of plasma with that of the cisternal cerebrospinal fluid for a number of natural and administered ions and molecules. The results are shown in Table I. It is obvious that there are large differences between the concentrations in the dialysate which was considered to be in Donnan equilibrium with the plasma and those in the cerebrospinal fluid.

TABLE I

Substance	Plasma	csf	R_{csf}	R_{dial}
Na	148	149	1.005	0.945
K	4.3	2.9	0.675	0.96
Mg	2.02	1.47	0.92	0.80
Ca	5.60	2.47	0.45	0.65
Cl	106	130	1.23	1.04
HCO_3	25	22	0.42	1.04
^{82}Br	1	0.71	0.71	0.96
^{131}I high	13	2.6	0.20	0.85
low	0.25	0.0025	0.01	0.85
^{14}CNS high	8.25	4.50	0.545	0.73
low	1.77	0.071	0.04	0.60
Glucose	8.3	5.35	0.64	0.97
Urea	8.35	6.5	0.78	1.00
Osmolality	298.5	305.2	1.02	0.995
pH	7.46	7.27	-	-

Table I shows the concentrations of various solutes (m equiv/kg H_2O) in cerebrospinal fluid and plasma of the rabbit, and also the distribution ratios R_{csf} = concentration in cerebrospinal fluid over the concentration in plasma and R_{dial} = concentration in blood dialysate over concentration in plasma (Davson, 1956; Pollay, 1966).

The latter contained more Na^+, Cl^- and Mg^+ and less K^+ and Ca^{++} than the plasma dialysate. Also the concentration of markers such as I^-, Br^- and CNS^- administered intravenously appear in the cerebrospinal fluid in greatly different concentrations than in the plasma. One can conclude from these observations that the cerebrospinal fluid cannot be a simple ultrafiltrate of the plasma.

The cerebrospinal fluid investigated was collected from the cisterna after passing through the ventricle system. There is abundant evidence that during this passage the composition of the fluid changes. To make a more meaningful comparison fluid should be collected not from the cisterna but directly as it is formed by the plexus. Such an investigation was carried out by De Rougemont, Ames, Nesbett and Hofmann (1960), and by Ames, Sakanoue and Endo (1964) who collected the fluid formed by the choroid plexus in the lateral ventricles under oil. An analysis of this fluid showed that it did not differ from plasma ultrafiltrate in Cl and K concentration but contained more Na and Mg and less Ca. Also this more adequate investigation suggested thus that the cerebrospinal fluid is not a simple ultrafiltrate of the plasma. Active mechanisms have been demonstrated in the choroid plexus able to transport compounds from the cerebrospinal fluid into the plasma against a concentration gradient. Pappenheimer, Heisey and Jordan (1961) observed such a transport for phenolsulfonphthalein and Diodrast, Davson, Kleeman and Levin (1961) and Davson and Pollay (1963) for p-aminohippurate and finally Becker (1961), Welch (1962), Pollay and Davson (1963), Coben, Loeffler and Elsasser (1964) and Pollay (1966) for radioactive iodide

and thiocyanate. Such active processes will be able to change the composition of the fluid produced by the plexuses and may be partly responsible for the observed differences between this fluid and plasma ultrafiltrate.

The cerebrospinal fluid collected from the cisterna is a fluid formed by the choroid plexus, perhaps as an ultrafiltrate, modified by active transport processes in the plexus, and undergoing additional alterations during its passage through the ventricles and aqueduct.

Composition of the extracellular fluid of the brain.

In regions of the body other than the brain, such as e.g., skeletal muscle, the extracellular fluid is usually considered to be an ultrafiltrate of blood plasma, the factor favoring filtration being the hydrostatic pressure in the capillaries, and the opposing force being the colloid osmotic pressure of the plasma proteins. These conditions are not uniform, because the hydrostatic pressure causing filtration is relatively high at the arterial end of a capillary, while at the venous end it is lower, so that here the osmotic force may be the greater, leading to absorption of the extracellular fluid (Starling, 1896). This dynamic state leads to a slow turnover of the extracellular fluid, formation at the arterial end, absorption at the venous end of the capillary. Any excessive production is removed by the lymphatic system. The chemical composition of this extracellular fluid can be predicted on the assumption that it is an ultrafiltrate in Donnan equilibrium with the plasma. However, when a substance is involved in

the metabolism of the tissue such as glucose or phosphate, its concentration cannot be predicted. Insofar as these fluids have been analyzed, these assumptions were confirmed, even though the filtrate is far from perfect, containing quite a high concentration of protein. The mechanism whereby the protein concentration is kept within reasonable limits is through the operation of the lymphatic capillaries; their walls are highly permeable so that proteins can diffuse into them along with the other constituents of the extracellular fluid. The flow of extracellular fluid back towards the venous blood is maintained by virtue of the valves in the large lymph vessels.

Davson (1967) pointed out that so far as the central nervous system is concerned, a different mechanism for the maintenance of a suitable environment for the cells of the parenchyma must be postulated because of the absence of a lymphatic system. A continuous filtration of plasma by the capillary membranes, associated with reabsorption at the venous ends, would present serious problems caused by the leakage of protein since the protein would not be able to diffuse back into the plasma until its concentration in the extracellular fluid had become greater than that in the plasma. This problem had apparently been solved in two ways; first, the effectiveness of the capillaries as protein filters is increased by a general reduction in permeability, i.e. by the establishment of a blood-brain barrier; and secondly, by the employment of the cerebrospinal fluid as a drainage system, the ependymal and glial linings being sufficiently permeable to protein and other large molecules to allow a relatively unrestricted escape from the

tissue into the cerebrospinal fluid. The drainage into the blood would be similar in both the lymphatic and cerebrospinal systems, namely by flow through unrestricted channels. The cerebrospinal fluid after flowing through the ventricular system and taking up extracellular fluid on its way, passes through the foramina of Luschka and Magendie into the cisterna then flows over the hemispheres and finally drains through the arachnoid granulations into the sinuses. The presence of a blood-brain barrier, only little permeable to the small solutes of the plasma, let alone to its proteins, would be of value in insulating the central neurons from the effects of rapid fluctuations in the blood concentrations of certain ions, such as K^{+}, Mg^{++}, and Ca^{++} that affect the excitability of neurons and synapses.

The composition of the fluid which passes the blood-brain barrier is unknown. It may be a plasma ultrafiltrate. However, the possibility that the blood-brain barrier, just as the choroid plexuses, is the seat of active transport processes which can materially change the composition of the fluid passing into the extracellular space should not be overlooked. It has even been suggested that the fluid is formed by a process of secretion by the astrocytes lining the cerebral capillaries or by the endothelial cells of the capillaries themselves (Davson, 1956).

The concept that the extracellular fluid participates in the formation of the cerebrospinal fluid and the relatively unrestricted diffusion across the ependyma lining the ventricles has led to the suggestion that the ionic composition of the cisternal cerebrospinal fluid

is similar if not identical to that of the extracellular fluid. It was attempted to prove this similarity by investigating the composition of the cerebrospinal fluid drawn from different regions of the fluid systems. It could be expected that the longer a cerebrospinal fluid differing in composition from the extracellular fluid had been in contact with the brain the more it would resemble the latter. Differences in composition of fluids drawn from different regions would then indicate differences between solute concentrations in cerebrospinal- and extracellular fluids. Similarity in composition of cerebrospinal fluid drawn from different regions would argue for a similarity of the two fluids.

Davson (1958) reasoned that successive samples drawn from the cisterna magna can be expected to be derived from different regions of the fluid system. The first sample would represent fluid that has recently left the ventricles, whilst later samples presumably represent fluid from more remote regions such as that bathing the hemispheres. When such successive samples from the rabbit and cat were analyzed for Cl^- and glucose (Davson, 1958), urea (Kleeman, Davson and Levin, 1962) and potassium (Bradbury and Davson, 1965), no consistent differences in composition were found which seemed to indicate a similarity in composition of the cisternal cerebrospinal- and the extracellular fluids.

De Rougemont, Ames, Nesbett and Hofmann (1960) and Ames, Sakanoue and Endo (1964) compared the composition of the fluid freshly secreted from the exposed choroid plexus of the cat's lateral ventricle with that of the cerebrospinal fluid drawn from the cisterna magna. During

the passage of the fluid through the ventricular system the Cl^- concentration increased and the K^+, Ca^{++} and Mg^{++} concentrations decreased resulting in the typical composition of cisternal cerebrospinal fluid. As mentioned above these changes may be achieved by adding extracellular fluid to the fluid produced by the plexus and by an ion exchange between the cerebrospinal- and extracellular fluids through the ventricle lining. It would follow from this that the extracellular fluid has a higher Cl^- and a lower K^+, Ca^{++} and Mg^{++} concentration than the fluid produced by the plexuses in the lateral ventricles. If these processes are of sufficient magnitude it might indeed be assumed that the cisternal cerebrospinal fluid and the extracellular fluid are similar in composition.

The finding that cortical subarachnoidal fluid has an even lower K^+ concentration than the cisternal fluid (Bito and Davson, 1966) would indicate that the process of equilibration is not finished when the cerebrospinal fluid has reached the cisterna, or that the K^+ concentration in the cerebral cortex is even lower than in the tissue surrounding the ventricles. However, the K^+ concentration in the lumbar cerebrospinal fluid was larger than that of the cisterna. There is evidence (Van Harreveld and Ahmed, 1968) that when the cerebrospinal fluid reaches the subarachnoidal space it is not only altered by an interchange with the extracellular fluid but also by transudation and (or) diffusion of material through the wall of the pial vessels and by diffusion through the dura. The latter observation would make the use

of the ion concentrations in the subarachnoidal fluid inadmissable in discussions about the equality in ion composition of cerebrospinal and extracellular fluids.

The results of these studies suggested that the extracellular fluid is controlled by active processes which cause it to maintain a constant composition, differing materially from that of a plasma ultrafiltrate and from the fluid produced by the choroid plexuses. As mentioned above it has been suggested that this fluid is produced by astrocytes which contact the capillaries with their end-feet. They could transport the constituents of the plasma through their cytoplasm and out into the extracellular spaces, imparting to the fluid a specific composition (Davson, 1956). Another possibility is that an ultrafiltrate is formed which is modified by active processes at the blood-brain barrier. For instance the low K^+ concentration could be produced by an active transport from the extracellular fluid into the blood. It has alternately been suggested that the active processes are not situated in the blood-brain barrier, but are to be found in the choroid plexuses determining the composition of the cerebrospinal fluid. The latter would then impress a typical composition on the extracellular fluid by an interchange of material across the ependyma of the ventricles. This postulate which is known as the "sink action" of the cerebrospinal fluid will be discussed below.

The extracellular volume based on the composition of the cerebrospinal fluid.

If the extracellular fluid resembles the cerebrospinal fluid, then the ion concentrations of the latter and not those of the plasma should be used as the basis for an estimate of extracellular space in the brain. For instance the natural sulfate concentration of the cisternal cerebrospinal fluid in the rabbit is only about 1/6 of that in plasma (Van Harreveld, Ahmed and Tanner, 1966). The sulfate space of the brain is about 4% when computed on the assumption that the sulfate concentration in the extracellular fluid is similar to that of plasma (Woodbury, Timiras, Koch and Ballard, 1956; Barlow, Domek, Goldberg and Roth, 1961). However, when the sulfate space is computed on the assumption that the sulfate concentration in the extracellular fluid is similar to that of cerebrospinal fluid, it will be 6 times as large (or more than 20%) as when serum is used for this purpose (Van Harreveld, Ahmed and Tanner, 1966).

Streicher (1961) found that the thiocyanate space of central nervous tissue computed on the basis of the concentration of the marker in plasma water as the model for extracellular fluid varied from 4 to 17% depending on the thiocyanate concentration in the plasma. The ratio of the thiocyanate concentrations in cerebrospinal fluid and plasma water also varied with the plasma concentration of this compound (Streicher, Rall and Gaskins, 1964). At low concentrations of thiocyanate in the plasma water (below 1 mM/L) this ratio was very low but it increased with the plasma concentration to 0.8 to 0.85 at a

plasma concentration of 8 mM. At low concentrations, there is an active process in the choroid plexuses that removes thiocyanate from the cerebrospinal fluid (Welch, 1962; Pollay and Davson, 1963; Pollay, 1966). This active process is apparently saturated by increasing the thiocyanate concentration in the plasma. The dependence of the thiocyanate space of central nervous tissue on the dose administered may indicate that a similar relationship as that found between the thiocyanate concentrations in plasma and cerebrospinal fluid also exists for the extracellular fluid of the brain. The thiocyanate space, even at the highest estimates may still be low as an estimate of extracellular space since even at the highest plasma concentrations of thiocyanate the ratio of the concentrations in cerebrospinal fluid and plasma water was still less than one. A similar dependence of the iodide and bromide spaces on the plasma concentration has been found earlier by Weir and Hastings (1939), Weir (1942) and by Wallace and Brodie (1940).

From these considerations it would seem that the low estimates of the extracellular space in the brain based on the assumption that the concentrations of compounds such as sulfate, thiocyanate or iodide administered intravenously reach equality in plasma water and extracellular fluid are erroneous. The concentration in the latter may be closer but not necessarily equal to that of the cerebrospinal fluid. The estimates of the extracellular space based on this assumption are much higher, of the order of 15 to 20% of the brain volume.

The sink action of the cerebrospinal fluid.

Davson, Kleeman and Levin (1961) pointed out that because of the very low concentrations of compounds such as SO_4^{--}, CNS^-, and I^- in the cerebrospinal fluid, the latter would be able to act as a sink, continuously draining the tissue of these compounds which are transported over the blood-brain barrier from the plasma into the extracellular fluid. Thus, even after 24 hours during which the plasma concentration of the compound has been maintained constant, there would not be achieved a true equilibrium between plasma and brain extracellular fluid but rather a steady state with the concentration in the extracellular fluid lower than that in plasma, and closer to that in the cerebrospinal fluid. Davson and Bradbury (1965) suggested that if the concentration of the marker in the plasma is 100, and that in the cerebrospinal fluid is 2; the concentration in the extracellular fluid would achieve a steady state level between these two values, its magnitude could be 30 or less, depending on the permeability of the blood-brain barrier to the marker, its diffusion coefficient in the nervous tissue, the permeability of the brain-cerebrospinal fluid barrier, the rate of production of extracellular fluid, the rate of flow of cerebrospinal fluid, etc.

In order to make the sink action of the cerebrospinal fluid possible the concentration of the marker in the latter has to be low and a relatively free diffusion across the ependymal lining must be possible. Both requirements seem to be fulfilled for certain markers. For example, Morrison (1959) and Reed and Woodbury (1963) found that

the concentrations of inulin in the cerebrospinal fluid, 24 hours after an intravenous injection, was only 1 to 2% of that in plasma; there thus existed a steep concentration gradient between plasma and cerebrospinal fluid with the concentration in the extracellular fluid probably somewhere in between, enabling the cerebrospinal fluid to act as a sink for inulin. In a similar way, the ratio of the naturally occurring sulfate concentrations in cerebrospinal fluid and serum was only 0.17 in the rabbit (Van Harreveld, Ahmed and Tanner, 1966). Also in Barlow, Domek, Goldberg and Roth's study (1961) in which when $^{35}SO_4$ was administered the concentration in the cerebrospinal fluid after allowing 8 hours or more for equilibration was only 9% of that in the plasma. Rall, Oppelt and Patlak (1962) have shown that the exchange of inulin, which has a relatively large molecule, between cerebrospinal fluid and brain over the brain-cerebrospinal fluid barrier is relatively unrestricted.

Davson, Kleeman and Levin (1961) intimated that the extracellular fluid is formed as an ultrafiltrate with the same concentration of the marker as in the plasma which is then modified by the sink action of the cerebrospinal fluid. In this view the marker concentration in the extracellular fluid must be appreciably higher than in the cerebrospinal fluid to make diffusion through the spaces in the tissue and across the ependyma possible. As will be shown there is good evidence that similar active transport processes as found in the choroid plexus are also situated in the blood-brain barrier. The concentration of the marker in the extracellular fluid may therefore be much lower and may in effect approach that of the cerebrospinal fluid.

The extracellular volume of the brain based on a reverse sink action.

If the ependymal- and pia-glial membranes are, indeed, highly permeable membranes, allowing large inulin molecules (Rall, Oppelt and Patlak, 1962) and ferrocyanide ions (Allen, 1955) to diffuse across it could be reasonably expected that extracellular labels such as thiocyanate, sucrose and sulfate would pass with relative ease into the brain tissue when applied to these surfaces (reverse sink action). Extracellular tags could be introduced in this way into the extracellular fluid instead of by intravenous administration for an estimate of the extracellular space in central nervous tissue. The first attempt was that of Davson, Kleeman and Levin (1961), who repeatedly replaced the cerebrospinal fluid of rabbits with an artificial fluid containing a known concentration of labeled sucrose to maintain as far as possible a constant concentration in the subarachnoid spaces. After 2 to 3 hours the animal was killed and the central nervous system was analyzed. The sucrose space of the spinal cord was found to be 12 to 14% in this way, based on the assumption that the sucrose concentration in the extracellular material was equal to that of the fluid bathing the brain. Bourke, Greenberg and Tower (1965) injected ^{14}C-labeled thiocyanate, sucrose and inulin into the cisterna. The cisternal cerebrospinal fluid flows over the hemispheres to be removed through the arachnoid granulations. After a suitable time the label in the cerebral cortex was compared with the activity in the subarachnoidal fluid bathing this tissue. The thiocyanate space found in this way was similar to the chloride space, the sucrose and inulin spaces were slightly smaller.

The latter spaces were 19% of the tissue volume in guinea pigs, 22% in rabbits, 28% in cats and 33% in monkeys. Rall, Oppelt and Patlak (1962) perfused the ventricular system of the dog with an artificial cerebrospinal fluid containing ^{14}C-labeled inulin for 3 to 5 hours. The perfusion was by way of a cannula placed in the lateral ventricle and another in the cisterna magna (ventriculo-cisternal perfusion), a technique first developed by Royer (1950) and by Leusen (1950). At the end of the perfusion, the dog was killed and the brain removed. The 1 to 2 mm of tissue closest to the ventricle contained 5 to 15% of the inulin concentration of the perfusate, the next 2 to 3 mm contained 2 to 5%.

The results of experiments in which the extracellular label was introduced into the cerebrospinal fluid system yielded values for the extracellular space which are higher (12 to 33%) than when the label is administered intravenously. Nevertheless, this is not necessarily the true extracellular space. The system was not in equilibrium at the end of the perfusion period since the compound will be lost continually to the plasma through the blood-brain barrier, although in the case of compounds such as inulin, these losses may not be large due to the low permeability of the blood-brain barrier for them.

It has been proposed that simultaneous intravenous administration of an extracellular marker and perfusion of the ventricular system with an artificial cerebrospinal fluid containing the same concentration of this compound as the plasma would result in a concentration of the marker in the extracellular fluid equal to that in the plasma (or in the perfusate). Indeed the sucrose space which is not more than 3 to 4% when

the label is administered intravenously only (Davson and Spaziani, 1959; Reed and Woodbury, 1963) was 6% when determined by ventriculo-cisternal perfusion alone and 10% by the combined presentation through blood and cerebrospinal system (Oldendorf and Davson, 1967).

Active processes in the choroid plexuses.

The passage of $^{131}I^-$ from blood to brain and from blood to cerebrospinal fluid is restricted. When a constant concentration of the isotope is maintained in the blood for several hours, the concentration in the cerebrospinal fluid or brain-water is only a small fraction of that in the plasma (Wallace and Brodie, 1939; Davson, 1955; Davson and Spaziani, 1959; Reed and Woodbury, 1963). It was originally suggested by Davson (1959) and others, that bromide, iodide and thiocyanate were subjected to a detoxication process by the brain, being converted into substances which were able to pass the blood-brain barrier into the plasma to be rapidly eliminated from the blood. Their low concentration in brain and cerebrospinal fluid could be explained in this way. In fact, the "detoxication" process was shown by Pappenheimer, Heisey and Jordan (1961) to consist of an active transport of these substances from the cerebrospinal fluid to the plasma by the choroid plexus. They showed in experiments in which the ventricles and aquaduct were perfused in the goat, that Diodrast, with a molecular weight of 405, and phenolsulfonphthalein, with a molecular weight of 346, were absorbed by the choroid plexus from the perfusate much more rapidly than creatinine, with a molecular weight of 113.

Furthermore, the absorption of Diodrast occurred against a gradient when the concentration in the plasma was held above that in the perfusion fluid. The absorption seemed to be of a carrier mediated type since the percentage absorbed was reduced by increasing the concentration in the perfusate, and by competitive inhibition as it was shown that p-aminohippurate decreased the absorption of Diodrast.

Subsequent studies have confirmed that the slow passage of I^-, CNS^- and probably Br^- from blood to cerebrospinal fluid, and the very low steady state concentration observed in this fluid even after long periods, are due to active processes in the choroid plexuses which transport these ions against a gradient in the direction from cerebrospinal fluid to blood. This was demonstrated in the intact animal perfused from the lateral ventricles to the cisterna magna (Pollay and Davson, 1963; Reed, Woodbury, Jacobs and Squires, 1965; Coben, Gottesman and Jacobs, 1965; Bito, Bradbury and Davson, 1966). The active transport of iodide was also shown in the isolated choroid plexus by Becker (1961) and by Welch (1962). The iodide transport shows saturation as in a carrier mediated system and is inhibited competitively by p-aminohippurate (Pollay and Davson, 1963). Furthermore, perchlorate effectively inhibits the I^- transport when incorporated in the perfusion fluid or given intravenously (Becker, 1961; Coben, Loeffler and Elsasser, 1964; Bito, Bradbury and Davson, 1966). Metabolic poisons, such as dinitrophenol, p-chloromercuribenzoate and ouabain, inhibited the transport. This was observed *in vivo* (Davson and Pollay, 1963) as well as *in vitro* (Becker, 1961).

In the sink concept a significant factor in the maintenance of the low steady state level of $^{131}I^-$ and CNS^- in the brain is the low concentration of these ions in the cerebrospinal fluid by an active transport across the choroid plexuses, and by the constant renewal of the cerebrospinal fluid which removes the label passing the blood-brain barrier (Pollay, 1966; Reed, Woodbury, Jacobs and Squires, 1965; Bito, Bradbury and Davson, 1966).

Active processes in the blood-brain barrier.

The question arises as to whether there are similar active mechanisms operating across the blood-brain barrier as demonstrated for the choroid plexus, transporting iodide or other markers from the extracellular space into the blood stream. Such a mechanism could be an important factor in the maintenance of the very low concentration of $^{131}I^-$ found in the brain even after very long periods of time during which the isotope is kept at a steady level in the blood. Rall, Oppelt and Patlak (1962) argued that such a hypothesis is not necessary because of the ease with which solutes exchange between the cerebrospinal fluid and the brain extracellular fluid across the ependymal- and pia-glial membranes. The low concentration of $^{131}I^-$ in the cerebrospinal fluid, resulting from the active process in the choroid plexuses would in their opinion be sufficient to act as a drain on the adjacent tissue producing a steady state in which the concentrations in both cerebrospinal and extracellular fluids of the parenchyma are much lower than in the blood plasma.

To prove that there is an active process across the blood-brain barrier is difficult because of the exchange between cerebrospinal fluid and brain tissue. Attempts have been made to obtain information on this point by saturating the active processes. As mentioned above, Streicher (1961) investigated the thiocyanate space at different blood concentrations in rats and found that this space increases rapidly with increasing plasma concentration. In rabbits, Pollay (1966) demonstrated a similar relationship for CNS^- plasma concentration higher than 2 mM. In a similar way the $^{131}I^-$ concentrations in cerebrospinal fluid and brain have been measured at different plasma levels of unlabeled iodide. Bito, Bradbury and Davson (1966) showed an increase in the apparent $^{131}I^-$ space of rabbit brain and in the $^{131}I^-$ concentration in cerebrospinal fluid with plasma concentration. A maximal effect was obtained when the animal was in addition treated with perchlorate. Also, Reed, Woodbury, Jacobs and Squires (1965) showed the effect of increasing concentrations of unlabeled iodide on the $^{131}I^-$ concentration in the cerebrospinal fluid and on the I space in rats. The effects were clear-cut and demonstrated a dependence of the iodide space on the plasma level. These results were explained, without invoking an active transport across the blood-brain barrier in the following way. The raised plasma concentrations of iodide or thiocyanate will inhibit the active transport in the choroid plexuses and thus cause a rise in the concentration of the markers in the cerebrospinal fluid. This rise will reduce the losses of the labels from the extracellular fluid of the brain to the cerebrospinal fluid by a decrease of the concentration of the

gradient between these fluids, resulting in a higher concentration of the marker in the extracellular fluid and an increased iodide space. An alternate explanation of these observations would be that active transport processes which can be saturated operate both across the blood-cerebrospinal fluid and the blood-brain barriers.

To overcome this difficulty in interpretation, two main approaches have been employed by Bito, Bradbury and Davson (1966). In the first, the passage of iodide from blood into the spinal cord, isolated by a dural ligature from the main supply of cerebrospinal fluid, was studied in the presence and absence of inhibition of the iodide transport by perchlorate or of saturation with unlabeled iodide. Under these conditions the effect of the inhibitor or of saturation on the I^- concentration in the cerebrospinal fluid can not influence the concentration in the cord caudal of the ligature. The iodide spaces were determined in the cord above and below the ligature. Bito, Bradbury and Davson (1966) were of the opinion that the very small effects of added unlabeled iodide or of perchlorate observed on the I^- space of the cord under the ligature, made it unlikely that a carrier mediated transport system is of much, if any, significance in determining the low concentration of $^{131}I^-$ found after 6 hours of intravenous infusion. The question then can be asked as to how, in the spinal cord below the ligature where the sink action of the cerebrospinal fluid is excluded, such a low activity of $^{131}I^-$ is maintained. The authors proposed the unconvincing argument that the blood-spinal cord barrier is much less permeable for iodide than the blood-brain barrier, so that the penetration

from the blood is so slow that even after 6 hours no equilibrium between plasma and extracellular fluid is reached. Alternatively, as was proposed by Reed and Woodbury (1963) with respect to the brain, there might be a continuous flow of extracellular fluid from the tissue into the cerebrospinal fluid, which is drained away. If this extracellular fluid contained only a small fraction of the plasma $^{131}I^-$ concentration, a steady state would be reached with a low $^{131}I^-$ content of the tissue as a whole.

The second approach consisted of perfusing the ventricular system with an artificial cerebrospinal fluid containing $^{131}I^-$ and measuring the uptake by the brain in the presence and absence of perchlorate in the perfusion fluid. The apparent space of the brain in the steady state will depend on the rate of entrance across the ependyma and the rate of removal from the brain by the blood. If there is an active process transporting $^{131}I^-$ from the extracellular fluid of the brain to blood, the poisoned system should exhibit a larger I^- space. Bito, Bradbury and Davson (1966) perfused the ventricles with an artificial cerebrospinal fluid containing ^{131}I and perchlorate. The differences in the apparent $^{131}I^-$ spaces of brain, after 1, 2 and 3 hours of perfusion through the ventricles with and without perchlorate in the perfusion medium, were small and only statistically significant in the case of the 2 hour period. These experiments were believed to show that, if there is any active transport of $^{131}I^-$ across the blood-brain barrier, its influence on the iodide space of the brain during ventriculocisternal perfusion is very small. However, it is an assumption open

to doubt that an active process across the blood-brain barrier can be largely inhibited by perchlorate administered through the cerebrospinal fluid.

In the present investigation ^{131}I was used to examine the interrelationship between brain extracellular fluid, plasma and cerebrospinal fluid. The effectiveness of the active processes across the blood-cerebrospinal fluid barrier in transporting $^{131}I^-$ into the plasma was investigated. Finally the use of the iodide space as a measure of the extracellular space was considered.

METHODS

Rabbits of 3 to 4 kg weight, narcotized with pentobarbital were used. Radioactive $Na^{131}I$, in many experiments mixed with unlabeled iodide, was injected intravenously through the cannulated jugular vein. The renal vessels were ligated before the injection to prevent excretion of the iodide during the experimental period. Blood was collected by cannulating either the femoral or carotid artery. It was allowed to coagulate and after centrifugation the serum was used. Cerebrospinal fluid was obtained from the cisterna magna. The atlanto-occipital membrane was exposed and a needle, to which a thin polyethylene tube had been attached, was introduced through the membrane in a rostral direction. The orifice of the tube was placed about 10 cm below the needle to maintain a mild suction action on the cerebrospinal fluid. Only blood-free samples were used. The brain was removed by widely opening the brain case and transecting the medulla at the foramen magnum in addition to severing the brain nerves. The experimental animal was killed in a standard fashion by severing the abdominal aorta in order to remove as much blood from the brain as possible. The brain was collected 5 minutes later, weighed and homogenized in a mortar with 10 ml distilled water. The homogenate was transferred to a 25 ml volumetric flask and brought up to this volume. Also a piece of the quadriceps muscle was collected.

Iodide determinations. Serum and cerebrospinal fluid after suitable dilution were deproteinized with equal volumes of 8% trichloracetic acid (TCA). The radioactivity was determined in the clear supernatant in a well type gamma counter (Nuclear Chicago). In experiments

in which both labeled and unlabeled NaI were administered, the iodide concentration in the serum was determined chemically. The knowledge of the radioactivity and iodide concentration in the serum makes it possible to estimate the iodide concentration in other material such as brain and cerebrospinal fluid from their activity on the assumption that the ratio of labeled and unlabeled iodide is the same throughout the preparation.

The chemical determination was in principle a titration of iodine set free by a reduction of iodate by the iodide in the sample. This reaction was carried out at low pH by adding a surplus of iodate to the sample.

$$5\ I^- + IO_3^- + 6H^+ = 3I_2 + 3H_2O \qquad (3)$$

The iodine was removed by shaking with carbon tetrachloride ($C\ Cl_4$) in a separatory funnel. The violet solution of I_2 in $C\ Cl_4$ was collected. Usually 3 to 4 changes of $C\ Cl_4$ were sufficient to remove all the iodine. An iodide solution was added which became yellow due to the formation of tri-iodide ions ($I_2 + I^- = I_3^-$). This solution was titrated with $Na_2S_2O_3$. The tri-iodide ion (I_3^-) is the reactive ion and the equation involving the reaction of iodine should be written with I_3^- rather than I_2.

$$I_3^- + 2S_2O_3^{--} = 3I^- + S_4O_6^{--} \qquad (4)$$

The end point is indicated by the disappearance of the violet color of the $C\ Cl_4$ solution. Since 5 iodide ions produce 6 iodine atoms (3) which would need 6 thiosulfate ions in the titration, a 1 M iodide

solution is equivalent in this reaction with 6/5 mol of thiosulfate. In the computation the volume of the $Na_2S_2O_3$ solution times its molality had therefore to be multiplied by 5/6 to obtain the amount of iodide in the sample expressed in gram equivalents. The method was tested with iodide solutions. The iodide concentration of 0.015 M iodide solution was determined 8 times. The mean of these determinations was 0.0148 with a standard error of 0.00006 M.

About 1 g of brain homogenate and of the muscle were carefully weighed and the radioactivity was determined in the γ counter. From the brain weight and the dilution of the homogenate to 25 g the activity per gram brain was computed, neglecting the difference in specific weight of the brain and the homogenate.

Adsorption of iodide by the plasma proteins. Becker (1961), Reed, Woodbury, Jacobs and Squires (1965) and Cutler, Lorenzo and Barlow (1968) used the I concentration in plasma in computations of the iodide space. Reed and Woodbury (1963) used the TCA filtrate of the serum for this purpose. For the exchange of iodide between plasma and brain its concentration in the plasma water is the important value. Attempts were made to determine the adsorption of iodide on the plasma proteins.

Known amounts of iodide were added to normal rabbit serum. Several hours after the addition the iodide concentration was determined after precipitation with equal amounts of 8% TCA and removal of the proteins by centrifugation. To 9 ml serum 1 ml of 0.15 M NaI was added to a final serum concentration of 0.015 M. The mean of 7 determinations of the TCA filtrate was 0.0142 M with a standard error of 0.00008. Since

this diluted serum can be expected to contain about 6% protein, its water concentration is lower (about 94%) and the iodide concentration in the serum water should be greater ($\frac{100}{94}$ x 0.015 M = 0.0160 M) if all the iodide were present in the serum water. The difference of the expected iodide concentration and the actually determined concentration in the TCA filtrate is statistically significant ($p < 0.01$). It seems, therefore, that an adsorption of iodide to the protein removed in the TCA precipitate had taken place.

The adsorption of iodide ions on the serum proteins was more adequately investigated by mixing serum with labeled iodide either without or with unlabeled NaI. To 25 ml serum 5 μC of $Na^{131}I$ was added. Of this mixture 1.024 g was dried. This represents 1.002 ml serum, taking the dilution with the labeled iodide solution and the mean specific weight of rabbit serum (1.025, MacLeod, 1932) into consideration. The weight (and volume) of the evaporated water was 0.950 g; the weight of the protein 0.074 g. The serum water can thus be computed as 94.8 (vol) % and the weight of the protein was 7.2% of that of the serum. The count in 1 ml serum was 105,300 ^{c}/min. A volume of serum was mixed with an equal volume of 8% TCA and centrifuged. The count in 1 ml of the TCA filtrate was 50,800 ^{c}/min. If it is assumed that all the iodide dissolved in the serum water is present in the TCA filtrate then the 0.948 ml of water in the sample was diluted to 1.948 ml. The radioactivity of the serum water would then be $\frac{1.948}{0.948}$ x 50,800 ^{c}/min/ml or 104,400 ^{c}/min. The iodide adsorbed by the serum protein was thus 900 ^{c}/min/ml or less than 1% of the serum count.

A similar experiment was carried out after adding in addition to the labeled iodide, unlabeled NaI to a final concentration in the serum of 24 mM. The weight of the protein was 7.1% of that of the serum. The serum water was 95.1 (vol) %, and 4.7% of the iodide was adsorbed on the serum protein.

The TCA filtrate was used by Reed and Woodbury (1963) as a measure for the iodide concentration in serum water. It is possible, however, that either the precipitated proteins adsorb more iodide than the proteins in solution, or that iodide adsorbed on the serum protein is released during the treatment with TCA. It was therefore attempted to determine the iodide concentration in the serum water by dialysis as performed by Bito, Bradbury and Davson (1966) using the method of Davson, Duke-Elder and Maurice (1949). These authors dialyzed serum against a NaCl solution contained in a collodion sack until equilibrium was supposed to have been reached. It seems, however, that under the conditions of this experiment, in which the difference in osmotic pressure, caused by the presence of protein acting as a multivalent anion, in one of the compartments is not balanced by a hydrostatic pressure difference. No Donnan equilibrium will be reached but that the NaCl solution will eventually be transported into the serum compartment. Bito, Bradbury and Davson (1966) found after 16 hours of dialysis an iodide concentration in the dialysate indicating a binding of about 15% of the labeled iodide to serum protein. In their experiments on the iodide space they used a complicated system of continued dialysis to obtain

information on the I concentration in the plasma water during the experiment.

In the present investigation serum containing $Na^{131}I$ was dialyzed against a NaCl solution under constant agitation for 48 hours. As expected from the presence of protein in the serum a considerable loss of the NaCl solution was observed. The radioactivity of the TCA filtrate was compared with that of the dialysate. The count in the dialysate was 14.9% lower than that in the serum water computed from the TCA filtrate as shown above. In a second experiment in which both labeled and unlabeled iodide (to a final concentration of 24 mM) was added to the serum the count in the dialysate was 9.1% lower than that in the serum water.

The true iodide concentration in the serum water cannot be satisfactorily estimated by either of the two methods, since the TCA treatment may well alter the binding of iodide to the serum proteins, and the impossibility of reaching a true equilibrium under the conditions of the dialysis experiments makes the latter method suspect. In the present investigation the concentration in the TCA filtrate has been used as a measure for the iodide concentration in the serum water, although it has to be kept in mind that these values may be too large.

The determination of the percentage of blood in brain. The radioactivity of the blood in the brain vessels interferes with the determination of the radioactivity in brain tissue proper since blood had in all experiments a high activity in comparison with brain. One could remove the blood by perfusion of the brain, but it is possible

that in this way some of the radioactivity of the brain tissue is washed out. Therefore, the activity of the brain tissue proper was determined by subtracting the activity of the blood in the vessels from that of the brain.

The blood in the brain vessels was estimated using serum albumin iodinated with ^{131}I which is a large molecule and is unable to pass readily into the brain tissue. By comparing the activity in blood and brain, the volume of the blood in the brain vessels can be estimated. The labeled albumin was dialyzed at 4°C against an isotonic NaCl solution (0.9%) to remove iodide or other low molecular weight compounds. The radioactivity of the dialysate was monitored. It was found that after about one week most of these compounds were removed. Dialyzed ^{131}I albumin (about 100 μC) was administered intravenously. The times after which cerebrospinal fluid, blood, brain and muscle were collected, were 5 or 20 minutes. The rabbit was killed by the standard method described above. In some experiments cerebrospinal fluid was collected to make the conditions similar to the usual experiments. In other experiments, cerebrospinal fluid was not collected to compare the blood percentage of the brain in both cases. In addition to the activity of blood, brain and muscle, the activity of the blood TCA filtrate was determined. The latter can be considered as the activity due to iodide or other low molecular weight compounds. The mean activity of the TCA filtrate in 13 experiments was about 1% of that of the blood, that of the cerebrospinal fluid was 0.05% in 5 experiments.

The radioactivity of the brain equals the volume of blood in the brain times the blood radioactivity. Knowing the brain weight the blood volume per gram brain can thus be computed from the blood count. The presence of iodide (and perhaps other compounds) in the blood could complicate this estimate because iodide in contrast to iodinated albumin does enter the brain tissue. However, the radioactivity of the low molecular weight compounds is not more than 1% of the total blood activity and as will be shown in later experiments not more than a few per cent of the iodide concentration in the serum is found in the brain after such short periods of time. The radioactivity in the brain due to these compounds can thus be neglected.

Table II shows the volume of the blood in the vessels of the brain and of muscle determined 5 and 20 minutes after the administration of labeled albumin. A mean volume per gram tissue weight of 1.25% with a standard error of 0.08% was found when cerebrospinal fluid had not been collected. In experiments in which cerebrospinal fluid had been removed the mean volume was $1.82 \pm 0.06\%$. This difference which is statistically highly significant ($p < 0.01$) is perhaps not unexpected since the collection of the cerebrospinal fluid flowing from a tube ending 10 to 15 cm below the cisterna will result in a reduction of the pressure in the brain case which may lead to an increase in filling of the brain veins. The blood volume (1.25 and 1.82%) found agrees well with the figures reported by Bito, Bradbury and Davson (1966) for the rabbit (1.5%). They are considerably larger than Reed and Woodbury's (1963) values for the rat (0.60%). The blood volume in the muscle was

TABLE II

Volume of blood in brain and muscle expressed as a percentage of the tissue weight

Time after administration	CSF not collected		Time after administration	CSF collected	
	Volume in brain (%)	Volume in muscle (%)		Volume in brain (%)	Volume in muscle (%)
20	1.54	0.60	20	1.84	0.72
20	1.47	0.74	20	1.78	0.55
5	0.99	0.32	20	1.85	0.56
5	1.31	0.52	20	1.78	0.23
5	1.15	0.54	5	1.75	0.66
5	1.32	0.42	5	2.05	0.63
5	0.99	0.50	5	1.67	0.65
Means	$1.25 \pm 0.08^{*}$	$0.52 \pm 0.05^{*}$		$1.82 \pm 0.04^{*}$	$0.57 \pm 0.06^{*}$

* Standard error

much lower than that in the brain for both groups of experiments (0.52 ± 0.05% and 0.57 ± 0.06% respectively).

Technique for the perfusion of the rabbits ventricular system. The technique for the ventriculo-cisternal perfusion used was similar to that described by Pollay and Davson (1963). The perfusion fluid was delivered to the left and right lateral ventricles by a dual infusion pump (Harvard Apparatus, Inc.) at a rate of 0.05 ml/min. The fluid after flowing through the third ventricle and the aqueduct was passed through a cut in the atlanto-occipital membrane allowing a dependable drainage of the perfusate. The artificial cerebrospinal fluid described by Merlis (1940) was used as perfusion fluid.

To prepare the rabbit for perfusion the skull was exposed through a medial incision. Holes (1.0 mm in diameter) were drilled through the bone 8 mm caudal of the coronal suture and 9 mm lateral from the midline, a window was cut in the atlanto-occipital membrane and the head was fixed in a stereotactic instrument. Two cannulae with a diameter of 0.8 mm connected with the infusion pump could be lowered through the holes into the brain. A pressure manometer was connected with the inflow tubing so that continuous monitoring of the intraventricular pressure was possible. At the beginning of the experiment, the orifice of the inflow cannulae were placed at the level of the calvarium. The infusion pump was started and the pressure needed to deliver 0.05 ml/min of the perfusion fluid was noted. The cannulae were then lowered 9 to 10 mm into the brain tissue passing through the ventricles. The pressure in the inflow tubes rose after which the cannulae were very slowly retracted.

Their location in the ventricles was indicated by a drop in the inflow pressure. The depth was usually 7 to 8 mm from the surface of the calvarium. It was possible in this way to perfuse the ventricles for 4 to 5 hours with an occasional readjustment of the depth of the cannulae. The pressure measured with the manometers during perfusion was 0.5 to 1.5 cm lower than the pressure at the start of the experiment when the orifices of the cannulae were at the level of the calvarium.

At the end of the experiment the ventricle system was perfused for a few minutes with a solution of toluidine blue in the perfusion fluid, to stain the tissue bordering the ventricles and aqueduct. After removal the brain was cut in slices by a number of coronal sections allowing the inspection of the lateral and third ventricles and the aquaduct. Only the results on preparations were used in which both lateral ventricles were stained and the perfusion thus had been bilaterally successful.

RESULTS

Changes of the iodide space with time.

Figure 2 shows the changes in the iodide space and the I concentration in the cerebrospinal fluid expressed as a percentage of the concentration in the TCA filtrate of the serum over an 8 hour period after the administration of ^{131}I (50 μC) alone. Even after 0.5 hours there is an appreciable amount of ^{131}I in the brain which increases slightly over the ensuing 8 hours. Bito, Bradbury and Davson (1966) called the I space present shortly after the administration of ^{131}I the rapidly equilibrating space. This space is due partly to the blood remaining in the vessels. Since in all these experiments cerebrospinal fluid was collected, the mean magnitude of the vascular space can be estimated as 1.8% (indicated by the interrupted line in Fig. 2). This is still more than 1% smaller than the I space determined 0.5 hours after the iodide administration (3.2%). Bito, Bradbury and Davson (1966) ascribed this part of the rapidly equilibrating space to iodide present in the capillary walls and the glia surrounding these vessels. There are regions in the brain in which the blood-brain barrier is absent, such as the tuber cinereum (Rachmanow, 1913) and the area postrema (Wislocki and Putnam, 1920). Since dyes can enter these regions they should be accessible to the much smaller I ions. Part of the extravascular rapidly equilibrating space may therefore be due to the rapid penetration of iodide into these regions. Since the vascular space is the only clearly defined part of rapidly equilibrating space which is

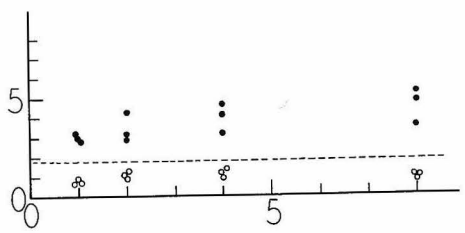

Fig. 2. Relationship between the time after administration of labeled iodide, the iodide space in the brain (points) and the iodide concentration in the cerebrospinal fluid, expressed as a percentage of the iodide concentration in the serum TCA filtrate (circles). The time in hours is plotted on the abscissa, the space and concentration on the ordinate. The interrupted line indicates the vascular space.

not part of the extracellular space of the brain parenchyma a correction has been made in the ensuing experiments for this entity.

The mean (corrected) iodide space increased from 1.6% after 1 hour to 2.1 and 2.6% after 4 and 8 hours respectively. The iodide in the cerebrospinal fluid had a value of about 1% of that of the serum TCA filtrate from 2 to 8 hours after the ^{131}I administration. These low values for the iodide space and the I content of the cerebrospinal fluid are a demonstration of the effectiveness of the blood-brain and blood-cerebrospinal fluid barriers which are able by the various processes considered in the introduction to keep the I concentration low in the brain extracellular- and cerebrospinal fluids.

Figure 3 shows the changes in the iodide space and the I concentration in the cerebrospinal fluid expressed as a percentage of that in the TCA filtrate of the serum in preparations in which in addition to ^{131}I (50 μC) unlabeled iodide was administered to a plasma concentration of about 15 mM. Again the interrupted line indicates a vascular space of 1.8% and the iodide space of the brain tissue can be seen to rise from a mean of 3.2% after 1 hour to 7.9% after 4 hours. The 8 hour figure is 7.4% which may not be different from the 4 hour value. From 8 to 16 hours the iodide space increases to a mean of 10.5% and becomes quite variable. A similar course has been observed with other labels with which the space reached a plateau after 4 to 5 hours, to increase slowly with longer intervals after the administration of the marker. For instance Woodbury, Timiras, Koch and Ballard (1956) administered labeled sulfate to rats. The sulfate space reached a value of 3.9% in

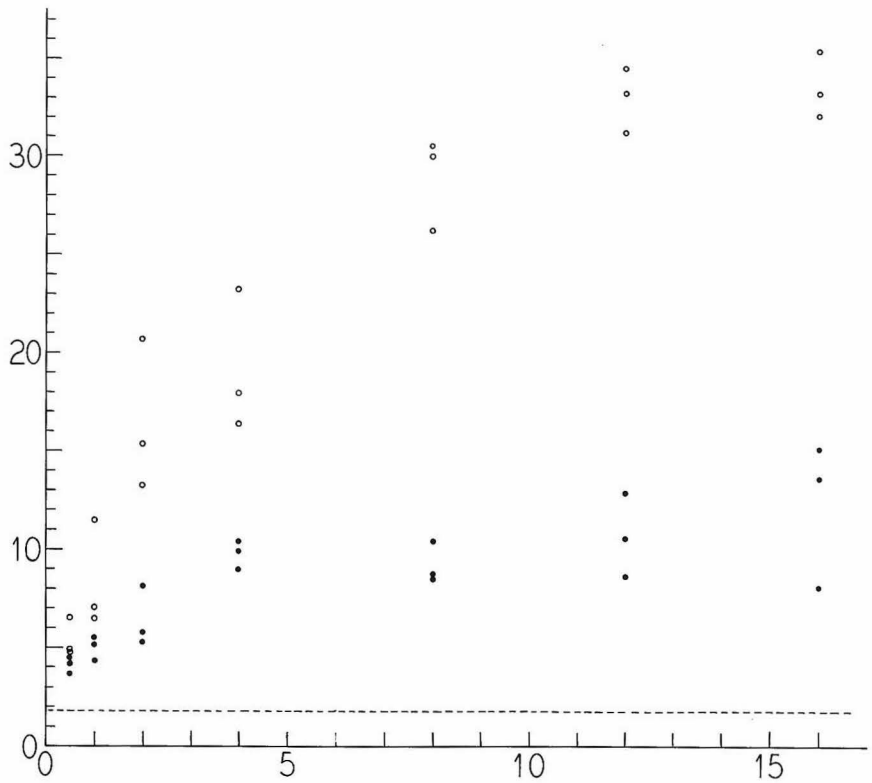

Fig. 3. Relationship between the time after administration of labeled and unlabeled iodide (to a plasma concentration of about 15 mM), the iodide space in the brain (points) and the iodide concentration in the cerebrospinal fluid, expressed as a percentage of the iodide concentration in the serum TCA filtrate (circles). The time in hours is plotted on the abscissa, the space and concentration on the ordinate. The interrupted line indicates the vascular space.

4 hours, stayed at this level for 16 hours and then increased to 32% in the ensuing 48 hours. This course has been interpreted by the assumption that after 8 to 16 hours the marker starts to penetrate into the cellular compartment. This interpretation is supported in the present experiments by the finding that the smallest iodide spaces after a 12 to 16 hour interval are not different from those in the 4 and 8 hour experiments. It could be surmised that in these instances the iodide had remained restricted to the extracellular space. It is for this reason that in the following experiments determinations were done 4 to 5 hours after administration of iodide.

The iodide content of the cerebrospinal fluid 1 hour after the administration of both labeled and unlabeled iodide is 8.3% of the TCA filtrate of the plasma to increase to a mean of 19.8% after 4 hours and to rise to values of 26.8%, 33.0% and 33.6% after 8, 12 and 16 hours respectively.

Iodide space as a function of the iodide concentration in the plasma.

Figure 4 shows the iodide space 5 hours after the administration of labeled iodide (50 μC) and varying amounts of unlabeled iodide, administered intravenously as a 0.5 M NaI solution. On the abscissa is plotted the iodide concentration in the plasma determined with the chemical method, on the ordinate the iodide space computed from the radioactivity in the brain tissue and that in the TCA filtrate of the serum as a measure of the labeled and unlabeled iodide. The interrupted line again indicates the vascular space. The (corrected) iodide space seems to

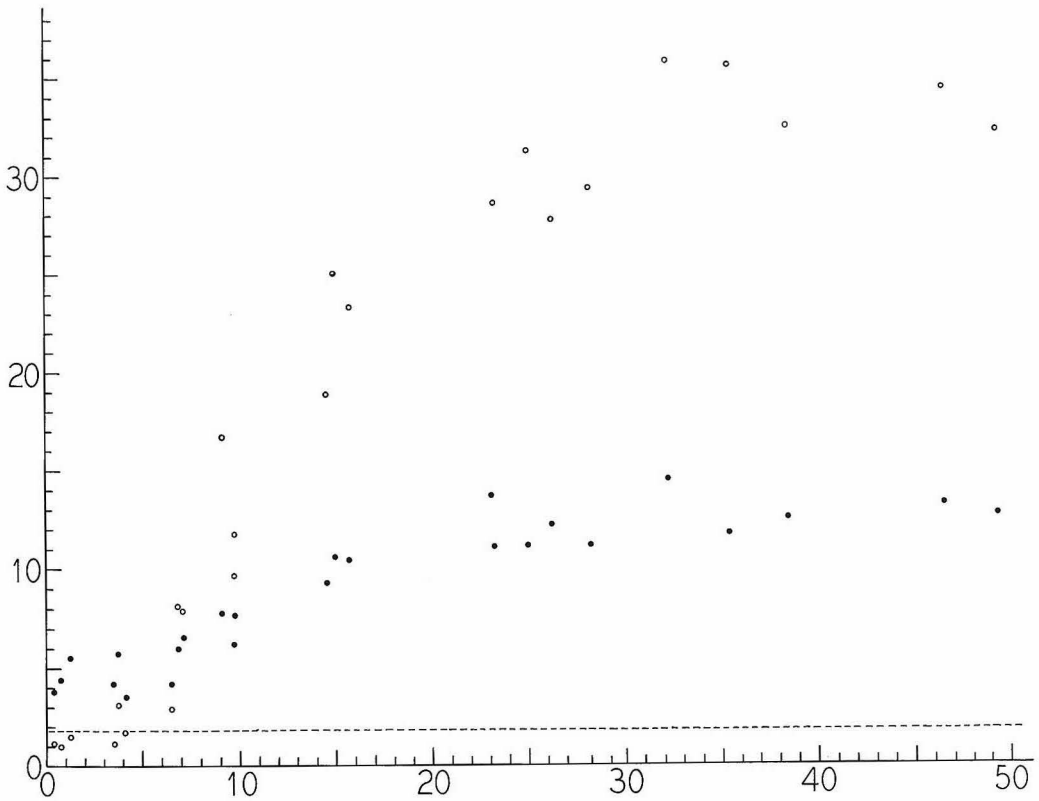

Fig. 4. Relationship between the plasma concentration of labeled and unlabeled iodide, the iodide space in the brain (points) and the iodide concentration in the cerebrospinal fluid, expressed as a percentage of the iodide concentration in the serum TCA filtrate (circles). The iodide concentration in the plasma is plotted on the abscissa, the space and concentration on the ordinate. The interrupted line indicates the vascular space. The plasma iodide concentration is expressed in mM/l.

remain constant at a mean of 2.7% for plasma iodide concentrations up to 5 mM. At higher plasma concentrations the space rises to reach a plateau at about 25 mM. The mean space at a serum I concentration of 25 to 50 mM is 10.6%. The constant space up to 5 mM may be explained by the observation of a threshold concentration in the plasma for iodide and thiocyanate below which the active processes are able to cope with the influx of these ions into the brain tissue transporting them back into the plasma (Wallace and Brodie, 1940; Streicher, Rall and Gaskins, 1964). Above the threshold concentration these active processes become more and more saturated as the plasma iodide concentration rises until at a concentration of about 25 mM saturation is complete and from that point the iodide concentration in the tissue increases proportionally with the plasma concentration. This is expressed as the constant iodide space at plasma concentrations higher than 25 mM.

Also the iodide concentrations of the cerebrospinal fluid expressed as a percentage of that of the serum TCA filtrate suggest a threshold value at concentrations of the plasma iodide below 5 mM. The iodide content of the cerebrospinal fluid rises quickly with that of the plasma at concentrations in the latter above 5 mM. The mean iodide concentration in the cerebrospinal fluid of the 4 experiments in which the serum iodide concentration was about 25 mM was 29.3% of that in the TCA plasma filtrate, to rise still a little when the plasma concentration was increased to 50 mM. However, even at the highest concentration of iodide in the plasma the concentration in the cerebrospinal fluid did not reach a value higher than about 1/3 of that of the plasma concentration.

In the concept of Bito, Bradbury and Davson (1966) the iodide concentration in the brain would be determined mainly by the sink action of the cerebrospinal fluid. The increase of the iodide space with increasing serum I concentration was explained by a rise in iodide content of the cerebrospinal fluid caused by the saturation of active transport in the choroid plexuses. However, it would seem possible that an active transport which in the present experiments was saturated with iodide is present also in the blood-brain barrier in addition to that in the choroid plexus.

Figure 5 shows the iodide space in muscle (quadriceps muscle) of the preparations used in Fig. 4 as a function of the iodide concentration in the serum. Obviously there is little effect of the plasma concentration on the iodide space. The interrupted line indicates the vascular space in the muscle (0.6%). The corrected mean iodide space of all the determinations was 9.6%. This figure is close to the value of the inulin space of muscle (9.6% found by Creese, d'Silva and Hashish, 1955, and 10.0% determined by Law and Phelps, 1966) which can be considered as a measure for the extracellular space. The iodide space would, therefore, at plasma concentrations up to 50 mM be a measure of the extracellular space, inferring that little or no iodide passes into the intracellular compartment of the muscle during the 5 hour duration of the experiment.

Iodide space in perchlorate treated preparations.

Perchlorate has been found to be an effective inhibitor of active processes transporting iodide. Bito, Bradbury and Davson (1966)

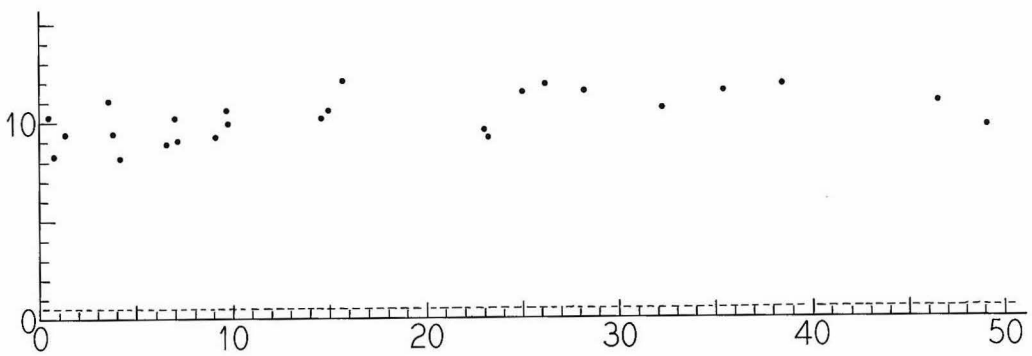

Fig. 5. Relationship between the plasma concentration of labeled and unlabeled iodide and the iodide space in muscle (quadriceps). The iodide concentration in the plasma is plotted on the abscissa, the space on the ordinate. The interrupted line indicates the vascular space. The plasma iodide concentration is expressed in mM/l.

showed that the inhibition in rabbits is immediate after a single intravenous administration of 3 mM perchlorate. In the present experiments 3 mM perchlorate was injected with the ^{131}I, then 1 mM of the inhibitor was injected hourly during the 5 hour period of the experiment, in an attempt to maintain inhibition at a constant level.

In Table III the data of 5 such experiments are collected. The iodide space after subtraction of the vascular space had a mean value of 8.2%, somewhat smaller than the space determined in preparations in which the active processes were saturated by a plasma iodide concentration of 25 mM and higher (10.6%). This difference was statistically not significant. The iodide concentration in the cerebrospinal fluid (mean 26.4%) also was somewhat smaller than that in the preparations treated with unlabeled iodide (29.3%). The values for the iodide space in muscle (mean 9.0) are statistically not different from those obtained in the latter preparations. A comparison of these results with those of experiments in which only ^{131}I was administered (Fig. 2) demonstrates the effect of perchlorate on the active processes and the resulting increase of the iodide concentrations both in the brain tissue and the cerebrospinal fluid. They show, furthermore, that the depression of the active processes by this compound also does not raise the iodide concentration in the cerebrospinal fluid to the plasma concentration. As in the experiments in which active transport was saturated by unlabeled iodide the cerebrospinal fluid concentration remained below 1/3 of the plasma concentration.

TABLE III

Iodide spaces in brain and muscle and iodide concentration in cerebrospinal fluid in preparations injected 5 hours previously with labeled iodide and perchlorate.

No.	$\frac{\text{brain}}{\text{serum}}$ x 100	$\frac{\text{csf}}{\text{serum}}$ x 100	$\frac{\text{muscle}}{\text{serum}}$ x 100
1	8.9	26.3	11.2
2	9.4	30.3	9.2
3	7.9	27.8	7.2
4	6.4	21.9	6.5
5	8.5	25.7	10.9
Means	8.2	26.4	9.0

The possibility was considered that the simultaneous administration of unlabeled iodide to a plasma concentration of about 25 mM and of perchlorate might further reduce active transport not completely eliminated by either procedure. Table IV shows the results of 5 experiments of this kind. The mean iodide space is somewhat higher (mean 12.4%) than in the experiments in which unlabeled iodide was administered alone (10.6%). This difference was statistically not significant. However, the iodide space after simultaneous administration of unlabeled iodide and perchlorate was considerably higher than after inhibition with perchlorate alone (8.2%). This difference was statistically significant ($p < 0.01$). The iodide concentration in the cerebrospinal fluid was also significantly higher (38.7%) than when unlabeled iodide or perchlorate had been administered alone.

Administration of labeled iodide by ventricle perfusion.

The effectiveness of the pathway into the brain extracellular space through the ependyma of the ventricular system was investigated by perfusing this system with artificial cerebrospinal fluid containing labeled iodide (5 μC/100 ml). The rate of perfusion was 0.05 ml per ventricle per min. The total amount of fluid flowing through the ventricular system (0.1 ml/min) was about 10 times that of the normal production of cerebrospinal fluid (Davson and Pollay, 1963; Pollay, 1967). Figure 6 shows the results of 9 experiments in which the system was perfused for 2, 4.5 and 7 hours. The mean iodide space in the brain computed as the ratio of the radioactivity in brain and in the

TABLE IV

Iodide space in brain and muscle and iodide concentration in cerebrospinal fluid in preparations injected 5 hours previously with labeled and unlabeled iodide to a plasma concentration between 25 and 30 mM and with perchlorate.

No.	$\frac{\text{brain}}{\text{serum}} \times 100$	$\frac{\text{csf}}{\text{serum}} \times 100$	$\frac{\text{muscle}}{\text{serum}} \times 100$
1	13.0	45.5	9.9
2	14.1	40.0	12.2
3	10.3	32.3	10.3
4	11.4	35.7	7.5
5	13.4	40.0	9.5
Means	12.4	38.7	9.9

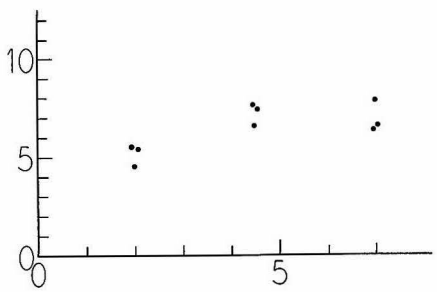

Fig. 6. Relationship between the time after the start of ventricle perfusion with artificial cerebrospinal fluid containing labeled iodide and the iodide space in brain. The time is plotted in hours on the abscissa, the space on the ordinate.

perfusion fluid (x 100) was 5.1% after 2 hours, 7.2% after 4.5 hours and 6.9% after 7 hours. No correction for the vascular space is necessary in these experiments since the iodide concentration in the blood of the non-nephrectomized rabbits remained low. These figures suggest that whereas after 2 hours the steady state has not been reached 4.5 hours are sufficient since the 4.5 and 7 hour values are statistically not different. The iodide space is materially larger than when iodide is administered intravenously (2.1 to 2.6%).

The iodide penetrating into the extracellular fluid of the brain from the artificial cerebrospinal fluid perfusing the ventricle is removed again by diffusion over the blood-brain barrier into the blood which at least at the start of the experiment is free of iodide. Also a hypothetical active transport mechanism in the blood-brain barrier moving iodide from the extracellular space into the blood would remove ^{131}I from the brain. After 4.5 hours of perfusion a steady state is achieved and the inflow and outflow of iodide are the same.

In the experiments in which the ^{131}I was administered intravenously the iodide diffuses through the blood-brain barrier in the direction from blood to extracellular fluid. Active processes in the barrier may transport part of the iodide back into the blood, but in addition the sink action of the cerebrospinal fluid will remove iodide. The higher iodide space observed after perfusion of the ventricles as compared with the space after intravenous administration will be due to the elimination of the sink action and to a greater permeability

of the cerebrospinal fluid-brain barrier than of the blood-brain barrier for iodide.

Simultaneous intravenous and ventricular administration of labeled iodide.

It has been suggested (Bito, Bradbury and Davson, 1966; Oldendorf and Davson, 1967) that the simultaneous administration of a marker by intravenous injection and by perfusion of the ventricles with an artificial cerebrospinal fluid containing the same concentration of the marker as present in the plasma would cause its concentration in the extracellular fluid to rise to the same concentration as that of the plasma (and of the perfusate). The data from such experiments would allow the computation of the extracellular space.

The experiment was carried out in the following way. Labeled iodide (150 μC) was injected into the jugular vein and 20 minutes was allowed for the distribution of the marker over the extracellular space of the animal. Some of the injection fluid was saved for the preparation of the perfusate. Then a blood sample was taken, preventing clotting with oxalate. After centrifugation the activity in the plasma was determined together with that of a suitable dilution with artificial cerebrospinal fluid of the solution of labeled iodide injected intravenously. On the basis of the counts in these fluids the latter was then further diluted to a concentration about 10% below that of the plasma. After 1 to 1.5 hours this process was repeated. In general the activity in the plasma had dropped 15 to 20%. The perfusate was then again adjusted to about 10% below the plasma level. This was

repeated once more after about 1.5 hours and the perfusate was adjusted to a value 5 to 10% below the expected plasma level at the end of the experiment.

Table V shows the results of 6 experiments of this type. The ^{131}I concentrations in the TCA filtrate of the serum at the end of the experiment and that of the perfusion fluid are similar (in most experiments within 5%). The mean iodide space after correction for the vascular space is 10.2%. This value is considerably larger than that determined in experiments in which the label was administered intravenously only (2.4%); it is also larger than that observed after ventricular perfusion alone (7.2%).

The experiments summarized in Figure 6 in which only perfusion of the ventricles with ^{131}I was carried out show that at the rate of perfusion used (10 times that of the cerebrospinal fluid normally produced) the choroid plexuses are unable to remove the iodide from the perfusate and that a transport of ^{131}I from the perfusate into the brain occurs. It was postulated that in this experiment iodide is removed from the brain both by diffusion across the blood-brain barrier and possibly by an active transport from the extracellular fluid to the plasma. The additional intravenous administration of ^{131}I would make the diffusion in this direction impossible, but would not greatly affect an eventual active transport in that direction.

TABLE V

Iodide spaces in brain and muscle after intravenous injection of labeled iodide and ventricular perfusion for 4.5 hours with the same concentration of ^{131}I as in the plasma.

No.	$\frac{\text{brain}}{\text{serum}} \times 100$	$\frac{\text{fluid}}{\text{serum}} \times 100$	$\frac{\text{muscle}}{\text{serum}} \times 100$
1	10.3	105	10.2
2	9.5	93	10.6
3	9.4	106	-
4	11.5	102	8.0
5	10.4	96	-
6	10.1	96	9.5
Means	10.2		9.5

Simultaneous intravenous and ventricular administration of labeled iodide in perchlorate treated preparations.

To counteract active transport, perchlorate in the doses mentioned above was administered to rabbits in addition to the simultaneous intravenous administration of labeled iodide and perfusion of the ventricles. The results of four such experiments are shown in Table VI. The mean iodide space in these experiments was 16.8%, materially and significantly ($p < 0.01$) larger than in the experiment in which ^{131}I was administered in the same way but no perchlorate was given (10.2%). It seems unlikely that the larger space in the perchlorate treated animals would be due exclusively to an effect of the inhibitor on the choroid plexuses which in the untreated preparations might take some iodide out of the perfusate even at the high rate of perfusing used (10 times that of the normal cerebrospinal fluid production). These experiments, therefore, suggest the existence of an active transport of iodide across the blood-brain barrier which is inhibited with perchlorate.

Simultaneous intravenous and ventricular administration of labeled and unlabeled iodide.

An alternate way of reducing or eliminating active transport is by the administration of unlabeled iodide in a concentration sufficient to saturate this process. The adjustment of the activity in the perfusate to that of the plasma was carried out as described above. The iodide solution injected intravenously contained both the labeled

TABLE VI

Iodide spaces in brain and muscle after intravenous injection of labeled iodide and perchlorate, and ventricular perfusion for 4.5 hours with the same concentration of ^{131}I as in the plasma.

No.	$\frac{\text{brain}}{\text{serum}} \times 100$	$\frac{\text{fluid}}{\text{serum}} \times 100$	$\frac{\text{muscle}}{\text{serum}} \times 100$
1	15.8	100	10.7
2	16.8	90	9.4
3	17.9	106	11.2
4	16.9	94	10.9
Means	16.8		10.5

(150 μC) and unlabeled (0.5 M) iodide. Some of this was saved and after dilution with artificial cerebrospinal fluid used for the perfusion. The counts in plasma, brain and perfusate are in this way a measure for both the labeled and unlabeled iodide.

Table VII shows the results of 7 experiments in which in addition to ^{131}I unlabeled iodide was administered to a plasma concentration between 20 and 40 mM, which as shown in Fig. 4 is sufficient to produce maximum inhibition. The perfusate had an iodide concentration which differed not more than 5% from that of the TCA filtrate of the serum. The mean iodide space computed on the basis of the TCA filtrate of the serum is 20.8%. It is of interest that the iodide space in these experiments is larger than in the experiments in which the active transport was depressed with perchlorate (16.8%). If the depression of the active transport were the same and if the iodide remained restricted to the extracellular space an equal value would be expected in these two series of experiments.

Changes in the perfusion fluid during its passage through the ventricles.

When perfusing the ventricles with a fluid containing iodide it can be expected that in the beginning of the experiment iodide is lost to the brain tissue. As the experiment progresses and the iodide concentration in the extracellular material increases this I uptake will decrease. If at the end of the experiment the I concentration in the perfusate and in the extracellular fluid were equal no uptake would occur and the iodide concentration in the inflowing and outflowing

TABLE VII

Iodide spaces in brain and muscle after intravenous injection and ventricular perfusion for 4.5 hours with the same concentration of labeled and unlabeled iodide as in the plasma.

No.	Iodide conc. of serum (mM)	$\frac{\text{brain}}{\text{serum}}$ x 100	$\frac{\text{fluid}}{\text{serum}}$ x 100	$\frac{\text{muscle}}{\text{serum}}$ x 100
1	25.6	20.1	98	9.7
2	26.8	23.1	95	11.2
3	20.8	19.5	96	-
4	25.2	20.4	98	9.3
5	27.5	19.9	100	12.1
6	35.0	21.1	101	9.7
7	39.6	21.4	97	8.1
Means	28.6	20.8		10.0

perfusate would be the same. In a number of experiments the iodide uptake from the perfusate was investigated by collecting the fluid from a pool in back of the spinal cord which could be reached through the window in the atlanto-occipetal membrane. Figure 7 shows the counts in the outflow as a percentage of the activity of the inflowing perfusate at different times during an experiment in which both ^{131}I and unlabeled iodide to a plasma concentration of about 25 mM was administered. Whereas at the start of the experiment about 15% of the iodide in the perfusate was lost this loss decreased as time went on until after 4.5 hours the count in the outflow was less than 0.5% lower than that of the inflowing perfusate. During the period that the latter perfusate was collected the iodide concentration of the inflowing perfusate was 95% of that of the TCA filtrate of the serum. Although the rather high rate of perfusion (10 times that of the normal cerebrospinal fluid production) may not allow a complete equilibration of the iodide concentrations in the perfusate and the extracellular fluid, it would seem that no great differences in iodide content of these fluids exists after 4.5 hours of perfusion. If this is so then the iodide concentration in the outflowing fluid would be more appropriate for a computation of the extracellular space than the TCA filtrate of the serum. The extracellular space computed on this basis (corrected for the vascular space) was 23.0%. Computed on the assumption that the iodide concentration in the extracellular space is equal to that of the serum TCA filtrate the space was 21.7%. It can be surmised that in this experiment a state of equilibrium between the iodide concentrations in the

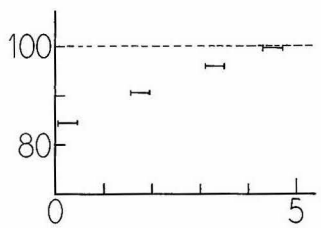

Fig. 7. Relationship between the duration of ventricle perfusion and the ratio of the radioactivity in the outflowing and inflowing perfusate (x 100). In this experiment labeled and unlabeled iodide was administered intravenously and the perfusate was adjusted to the iodide plasma level. The horizontal lines indicate the time needed to collect the outflowing perfusate. On the abscissa is plotted the time in hours, on the ordinate the ratio in activity.

ventricular perfusate, in the extracellular fluid and in the plasma water has approximately been reached, and that little or no diffusion across the blood-brain- and cerebrospinal-fluid-brain barriers takes place. If this is true then the iodide concentration in the plasma water is the same as that in the perfusate. The I concentration in the plasma water would thus be lower than in the TCA filtrate of the serum which would not be surprising in view of the dialysis experiments of plasma reported by Bito, Bradbury and Davson (1966) and those carried out in the present investigation.

Table VIII shows 5 such experiments in which the ventricles were perfused. The mean value for the extracellular space computed on the basis of the serum TCA filtrate is the same as that of the comparable experiments summarized in Table VII. With the exception of the last experiment, the outflow had a slightly higher count than the inflow. The perfusate had an activity of 87 to 95% of that of the TCA filtrate of the serum. Computed on the basis of the outflowing perfusate the mean extracellular space was 22.5%. This may be the best estimate of the iodide space in the brain.

TABLE VIII

Iodide spaces in brain and muscle after intravenous injection and ventricular perfusion for 4.5 hours with the same concentration of labeled and unlabeled iodide as in the plasma. Collection of the outflowing perfusate.

No.	$\frac{\text{brain}}{\text{serum}} \times 100$	$\frac{\text{fluid}}{\text{serum}} \times 100$	$\frac{\text{outflow}}{\text{inflow}} \times 100$	$\frac{\text{brain}}{\text{outflow}} \times 100$	$\frac{\text{muscle}}{\text{serum}} \times 100$
1	20.4	87	102	22.9	10.8
2	19.3	88	102	21.6	11.3
3	20.4	91	101	22.3	11.2
4	22.5	92	101	22.9	10.6
5	21.7	95	100	23.0	9.6
Means	20.8			22.5	10.8

DISCUSSION

Although the presence of an active transport mechanism in the choroid plexus is generally admitted, the existence of such a process across the blood-brain barrier is in doubt. Rall, Oppelt and Patlak (1962) suggested that the diffusion across the brain-cerebrospinal fluid barrier is sufficient to explain the low steady state concentration in the brain of ^{131}I administered intravenously. The increase of the iodide and thiocyanate space in the brain when the active transport is saturated by administering unlabeled iodide and thiocyanate in addition to the radioisotopes was explained by their effect on the choroid plexus and thus on the sink action of the cerebrospinal fluid rather than on an effect on an active transport in the blood-brain barrier. The experiments of Bito, Bradbury and Davson (1966) on the action of perchlorate and saturation with unlabeled iodide on the ^{131}I content of the spinal cord isolated by a dural ligature showed only a small effect on the iodide content of the isolated part of the cord where obviously no sink action was possible. The conclusion was drawn that active transport across the blood-brain barrier, if present at all, is insignificant. The low iodide content of the isolated cord was explained by the assumption that the blood-spinal-tissue barrier is much less permeable than the blood-brain barrier and that after 6 hours no equilibrium is reached. Alternate explanations are possible. The blood-brain barrier could produce an extracellular fluid of low ^{131}I content which is drained in the fluid surrounding the cord and carried

away through the granulations in the root veins (Reed and Woodbury, 1963).

Another experiment which seemed to show that active transport across the blood-brain barrier is of little importance was carried out by Bito, Bradbury and Davson (1966). They perfused the ventricles with a fluid containing ^{131}I in the same concentration as in the plasma with and without perchlorate. Also in these experiments in which the perfusion was continued for 2 to 3 hours, the difference caused by perchlorate was small. These experiments are in contrast to the present series in which the perchlorate was administered for 4.5 hours by intravenous injection. The preparations in which the ventricles were perfused with an artificial cerebrospinal fluid containing the same ^{131}I concentration as in the plasma, but in which no perchlorate was administered had an iodide space of 10.2%, those in which perchlorate was injected showed a space of 16.8%. This difference which is significant ($p < 0.01$) seems to indicate that an appreciable active transport across the blood-brain barrier is present.

The results obtained by Bito, Bradbury and Davson (1966) may be due to the difference in the manner of administration of perchlorate in addition to the longer time of perfusion in the present experiments. An alternate explanation of these results would be that in the experiments in which no perchlorate was administered the choroid plexus is able to remove so much ^{131}I from the perfusate that this now becomes able to act as a sink. Although this seems highly unlikely in view of the high rate of perfusion of the ventricles (10 times that of the

normal production of cerebrospinal fluid) an experiment was performed in which the ^{131}I concentrations in the inflowing and outflowing perfusate were determined. In this experiment which is schematized in Fig. 8, the ^{131}I concentration of the inflowing fluid after 5 hours perfusion was considerably higher than that of the TCA filtrate of the serum (112%) which may account for the rather large iodide space (12.8%) found in this instance. Assuming that the actual extracellular space is about 20% and contains all the iodide then the I concentration in the extracellular material can be estimated as 64% of that of the TCA filtrate of the serum. No diffusion from the extracellular space to the serum can therefore take place even if the I concentration in the plasma water is lower (for instance 90%) than that in the TCA filtrate. The concentration of the incoming fluid had a concentration of 112% of that of the TCA filtrate; the outflowing perfusate of 88%. Therefore, the extracellular compartment could also not lose iodide to the perfusate; on the contrary iodide can be expected to diffuse into the brain both through the blood-brain and through the cerebrospinal fluid barriers. In spite of this the iodide concentration in the extracellular fluid remained considerably below that of the perfusate and of the plasma. These observations support the concept of an active transport across the blood-brain barrier which moves iodide from the brain back into the plasma against a concentration gradient. A second experiment carried out in the same way produced similar results.

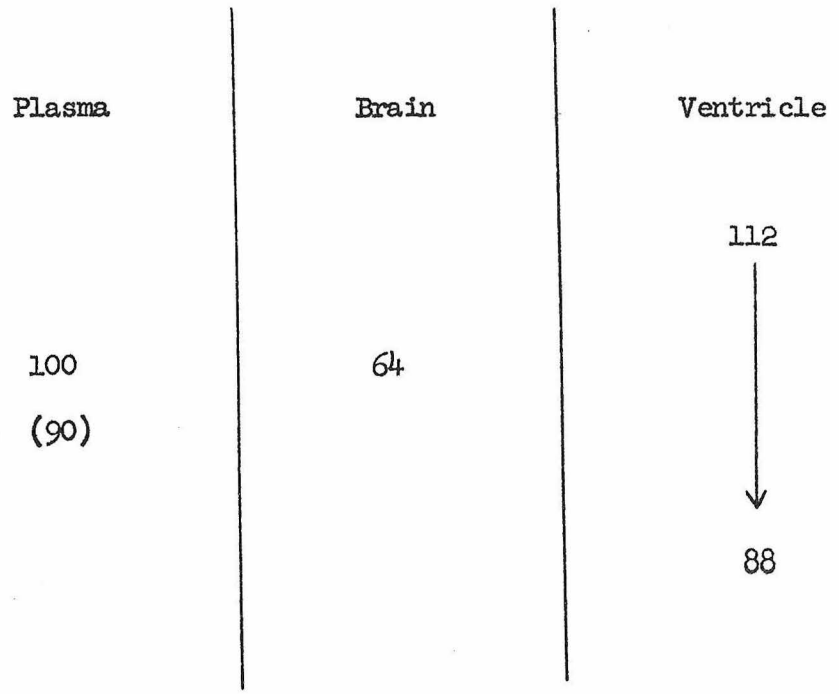

Fig. 8. On the left is the vascular compartment. The brain is represented by the middle- and the ventricles by the right compartment. The I concentration in the TCA filtrate is set at 100. The concentration in the plasma water may be lower, for instance 90. The I concentration in the extracellular fluid of the brain was computed as 64. The inflowing perfusate had an iodide concentration of 112, the outflowing fluid of 88.

When the sink action is prevented by perfusion of the ventricles with an artificial cerebrospinal fluid containing iodide in a similar concentration as present in the plasma and the active transport across the blood-brain barrier is inhibited by perchlorate or the administration of unlabeled iodide then the I concentration in the extracellular fluid can be expected to rise, approaching the plasma concentration. There was a difference between the iodide spaces (computed on the basis of the TCA filtrate of the serum) when the active transport across the blood-brain barrier was inhibited with perchlorate or saturated with unlabeled iodide. In the first instance the I space was 16.8%; in the second 20.8%. This difference which is statistically significant ($p < 0.01$) could be explained as a more effective inhibition of the active processes by saturation than by perchlorate inhibition. Also in experiments in which the ventricles were not perfused the saturation with unlabeled iodide yielded a higher iodide space (10.6%) than inhibition with perchlorate (8.2%). This difference was statistically not significant, however. Although perchlorate has been shown convincingly to inhibit active transport in the choroid plexus both in vivo and in vitro (Welch, 1962; Pollay and Davson, 1963), it may have a smaller effect on the active transport in the blood-brain barrier. In addition to the possibility that the transport processes in the choroid plexus and blood-brain barrier are not equivalent and thus may be affected by perchlorate differently, it would seem possible that perchlorate does not readily reach the locus of active transport in the blood-brain

barrier or that this very reactive compound is destroyed in the brain tissue.

On the other hand one could consider the possibility that the difference in space by perchlorate inhibition and unlabeled iodide saturation is due to the administration of hypertonic iodide solution in the latter instances which might change the magnitude of the extracellular space. To establish a plasma concentration of 25 mM about 20 ml of a 0.5 M NaI solution has to be administered per kg bodyweight. Due to the ready permeability of the cell membranes for water this will cause in a matter of minutes an osmotic equilibration due to water transport. Assuming that the water content of the rabbit is 70% then 20 ml of an osmolarity of 1 M are distributed in 700 ml of body water with an osmolarity of 0.3 M. The final osmolarity will then become 0.32 M, an increase of 7%. Such a change would not produce major changes in the iodide space of the brain since both the extra- and intracellular compartments are involved in the osmotic equilibration. The water movement can be expected to be followed by an ion exchange between the extracellular fluid of the brain and the plasma. Iodide will diffuse into the brain, replacing chloride moving into the plasma. If the rate of diffusion of these ions is not equal (and as will be discussed below, there are reasons to believe that this is so) then a change in the magnitude of the extracellular space could ensue. However, the constancy of the iodide space with plasma concentrations ranging from 25 to 50 mM indicates that this is not a significant factor since at an iodide plasma concentration of 50 mM a greater effect on

the magnitude of the extracellular space could be expected as at a concentration of 25 mM.

Assuming that the active transport both in the choroid plexus and in the blood-brain barrier is effectively suppressed by saturation with unlabeled I, then the only way in which iodide can be removed from the brain in experiments in which ^{131}I and unlabeled iodide were administered intravenously is by the sink action of the cerebrospinal fluid. In these experiments the mean iodide space was 10.6%. If the extracellular space is estimated as 20% and contains all the iodide then the I concentration in the extracellular material would be 53%. Since in these experiments the iodide concentration in the cisternal cerebrospinal fluid was between 30 and 35% of the serum TCA filtrate value, sink action was indeed possible. This action seems to be quite effective since elimination of the sink effect by perfusing the ventricles with an artificial cerebrospinal fluid containing an equal concentration of iodide as in the plasma almost doubles the I space (to 20.8%).

It is of interest that saturation of the active transport by administration of unlabeled iodide to serum concentrations of from 25 to 50 mM yields a constant I space. This means that the iodide concentration in the brain increases in proportion to the plasma concentration. Assuming that the extracellular space remains approximately constant and the rate of production of the extracellular- and cerebrospinal fluids remains the same at these iodide concentrations in the plasma, then the constancy of the iodide space might indicate that in the steady state the amounts of iodide flowing through the various barriers

are proportional to the plasma concentration. This would be in accord with the postulate that under these conditions iodide is transported only by diffusion, which can be expressed by Fick's equation.

$$D = K (C_1 - C_2)$$

This equation shows that the amount of material passing through a barrier (D) is proportional to the concentration gradient $(C_1 - C_2)$. The independence of the iodide space from the plasma I concentration (above 25 mM) thus supports the assumption that administration of unlabeled iodide effectively eliminates the active transport.

It is of considerable interest that at iodide concentrations in the plasma ranging from 25 to 50 mM the I concentration in the cerebrospinal fluid does not rise above about 1/3 that of the plasma concentration. If the assumption that active transport in the choroid plexus is eliminated is correct, then an explanation must be sought in the processes of filtration and diffusion by which the cerebrospinal fluid is formed under these conditions. Davson (1956) has considered the possibility that differences in ionic composition of plasma water and cerebrospinal fluid could be caused by molecular filtration. He rejected this possibility on the ground that the hydrostatic pressure differences between blood plasma and cerebrospinal fluid are insufficient to account for such a process. In Davson's concept of filtration the membrane would pass water and hold back part of the solutes, resulting in an osmotic difference between filtrate and filtrant. Indeed with the pressure available in the capillaries no significant concentration differences

could be expected. It would seem, however, that a restricted passage of certain ions or molecules through membrane pores during filtration could give rise to a difference in composition of filtrate and filtrant without a significant difference in osmotic pressure. Pappenheimer (1953) discussed such mechanisms in detail with respect to the passage of solutes across the capillary wall. Iodide seems to pass this barrier apparently unimpeded. However, the equivalent pores of the capillary wall (30 Å) are much larger than those of the rabbit blood-brain barrier which were estimated by Fenstermacher and Johnson (1966) as 7 to 9 Å. Assume that plasma containing as its main anions Cl^- and I^- is filtered through the pores of a membrane and that the I ions by their physicochemical properties are more restricted in their passage through the pores than the Cl ions. A filtrate could be obtained by such a mechanism in which the Cl^- concentration is greater, the I^- concentration smaller than in the filtrant without difference in osmolarity of these fluids. Since the dimensions of the hydrated Cl^- and I^- ions are very similar it seems unlikely that size would be the basis for such a difference in permeability. However, differences in the water shell could conceivably produce differences in the passage of these ions through charged pores. Such a filtrate may be further modified by diffusion through the pores as proposed by Pappenheimer (1953), evening out any osmotic differences by water movements and decreasing the concentration differences of Cl^- and I^- on both sides of the membrane. The final constitution of the filtrate would depend, therefore, not only on the restriction of the ion movements in the

membrane pores but also on the rate of filtration. If the latter is large the filtrate can be expected to show larger differences in Cl^- and I^- concentration than when the filtration is slow in which case diffusion tends to equalize the concentration differences.

It is of interest that saturation of active transport of thiocyanate results in a much higher concentration of this compound in the cerebrospinal fluid than iodide under the same circumstances. About 85% of the plasma level was found at a serum concentration of 7 mM (Streicher, Rall and Gaskins, 1964). Obviously the restricted passage of different ions through the membrane pores will depend on their physicochemical properties and may differ greatly.

The following summary of the processes involved in the distribution of iodide in brain and cerebrospinal fluid incorporates the observations made. The cerebrospinal fluid is formed by a process of molecular filtration and diffusion resulting in a fluid of materially lower I concentration than present in the plasma. The choroid plexuses can remove from this fluid a limited amount of iodide. This results in a very low iodide concentration of the cerebrospinal fluid when only ^{131}I is administered but a much larger iodide content when in addition unlabeled iodide is given. On its way through the ventricles the cerebrospinal fluid takes up iodide from the extracellular fluid (sink action). After passing through the cisterna the cerebrospinal fluid may take up additional iodide derived from the pial vessels and from the dura, and is finally removed through the arachnoid granulations. The extracellular fluid may be formed as a filtrate across the

blood-brain barrier, containing less iodide than the plasma water. Since the total surface of the brain capillaries is probably much greater than that of the vessels in the choroid plexuses the filtration rate across the blood-brain barrier may be smaller than across the choroid plexus (assuming that the fluid formed by the plexuses is a substantial part of total amount of cerebrospinal fluid produced). Diffusion may in that case wipe out to a great extent a difference in I^- concentration of filtrate and filtrant across the blood-brain barrier. An active transport process removes iodide from the extracellular space back into the plasma. The iodide moves partly by bulk transport, partly by diffusion (or more active processes such as movements of cellular elements; Pomerat, 1951) to the brain-cerebrospinal fluid barrier and there diffuses into the cerebrospinal fluid. About 5 hours after the iodide administration a steady state is reached in which the concentrations in the various compartments remain more or less constant.

By perfusion with an artificial cerebrospinal fluid containing a similar iodide concentration as present in the plasma and by administering ^{131}I and unlabeled iodide to a serum concentration of 25 mM or larger an iodide space of about 20.8% was found when computed on the basis of the TCA filtrate of serum. The I space was slightly larger (22.5%) when computed on the basis of the outflowing perfusate. The question can be asked in how far this space is a measure of the extracellular space. Two conditions have to be fulfilled if the iodide space is to be equivalent with the extracellular space. The iodide concentration in the extracellular fluid has to be equal to that of the plasma

(or to the fluid with which the ventricles are perfused) and the iodide has to remain restricted to the extracellular space. The similarity in iodide concentration of the inflowing and outflowing perfusate suggested that its concentration is close to that of the extracellular fluid in experiments in which both labeled and unlabeled iodide was used and the marker was administered intravenously in addition to the ventricular perfusion. The second condition that the iodide remains restricted to the extracellular compartment cannot be proven. However, such an assumption is supported by the iodide space in the muscle. With iodide concentrations in the plasma ranging from traces when ^{131}I only was used to 50 mM the extracellular space was constant and had a value of about 10%. This value agrees very well with the inulin space in rat muscle of 9.6% determined by Creese, d'Silva and Hashish (1955) and of 10.0% by Law and Phelps (1966). Since the space determined with inulin can be considered as a measure of the extracellular space it can be surmised that in the muscle at least no significant amounts of iodide penetrates into the intracellular compartment during the experiment. Obviously one cannot be sure that the brain tissue behaves as the muscle. However, there are other observations which support a large extracellular space in brain tissue. By applying markers in the subarachnoidal cerebrospinal fluid Bourke, Greenberg and Tower (1965) determined sucrose and inulin spaces in the rabbit's cortex of 22.5%. Saturating active transport by creating a high thiocyanate concentration in the plasma resulted in a thiocyanate space of 17%. Since the cerebrospinal fluid still had a thiocyanate concentration of 85% of that in the

plasma water, this space is probably too small (Streicher, 1961; Streicher, Rall and Gaskins, 1964). The sulfate space when computed on the assumption that the sulfate concentration in the extracellular fluid equals that in the cerebrospinal fluid is of the order of 20% (Van Harreveld, Ahmed and Tanner, 1966). The chloride space which in the rabbit is 32% (Davson, 1956) becomes 22% when a correction is applied for the high chloride concentration in the cerebrospinal fluid, and for the intracellular chloride (Van Harreveld, 1966). Furthermore, the specific impedance of cerebral tissue supports an extracellular space of 20 to 25% (Van Harreveld, 1966). Finally the use of special fixation methods (freeze-substitution and freeze-drying) have made it possible to demonstrate an appreciable extracellular space in electron micrographs of the cerebral and cerebellar cortex (Van Harreveld, Crowell and Malhotra, 1965; Van Harreveld and Malhotra, 1966, 1967).

Although in all estimates of the extracellular space certain assumptions have to be made it would seem that the evidence now available suggest a large space, of the order of 20% of the tissue volume.

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