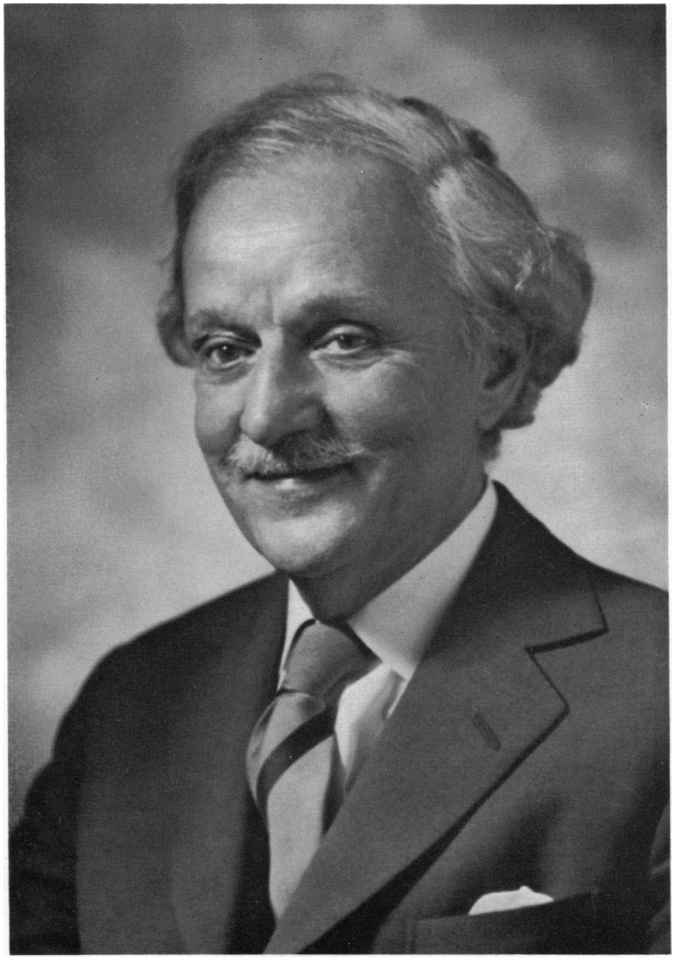

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J. Physiol. (1976), **255**, *pp.* 1–28
With 1 *plate and* 16 *text-figures*
Printed in Great Britain

REVIEW LECTURE

THE BLOOD–BRAIN BARRIER

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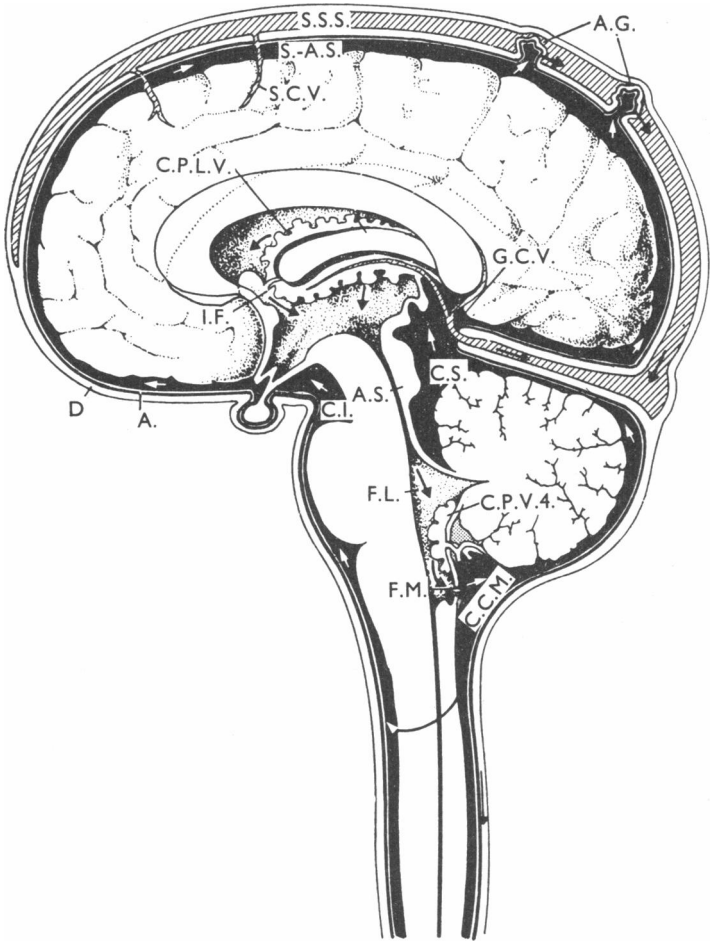
Early history. The concept of an impediment to the passage of dissolved material from the blood into the tissue of the brain and spinal cord, embodied in the term blood–brain barrier, derives from the early pharmacological studies of a number of German investigators who injected dyes into the blood stream and examined their distribution throughout the body, or else noted any pharmacological action. The general conclusion derived from such studies by Ehrlich was that practically all acid dyes failed to stain the grey matter of the brain whereas a number of basic dyes were able to do so. However, he attributed this failure not to an inability of the dyes to leave the blood stream but to a characteristic of the nervous tissue that prevented it from taking them up in amounts sufficient to become visible. By contrast, earlier workers such as Biedl & Kraus (1898) and Lewandowsky (1900), who studied the effects of I.V. injections of bile acids and ferrocyanide, attributed the absence of pharmacological effects when these were administered I.V. to a failure of the substances to leave the blood vessels, i.e. it was due to a barrier between blood and the tissue it nourished. Their belief was fortified by the finding that, when the substances were administered into the cerebrospinal fluid (c.s.f.), they had strong pharmacological effects. This difference between the results, according as the substance was injected into blood or c.s.f., was emphasized more emphatically by Goldmann (1909, 1913), who performed what have come to be known as his 'First' and 'Second Experiments'. The first experiment established that, on I.V. injection, the acidic dye trypan blue distributed itself widely throughout the body, but the brain and spinal cord remained virtually unstained, although the choroid plexuses were an exception. In his second experiment, he injected the dye into the c.s.f. and found definite pharmacological symptoms that had been lacking after injection by the I.V. route, whilst the nervous tissue was heavily stained, including the leptomeninges. Goldmann was impressed by the similarity between the placenta and choroid plexuses, having observed that injection of trypan blue into the maternal circulation left the foetus unstained although the placenta took up dye, and he considered that the

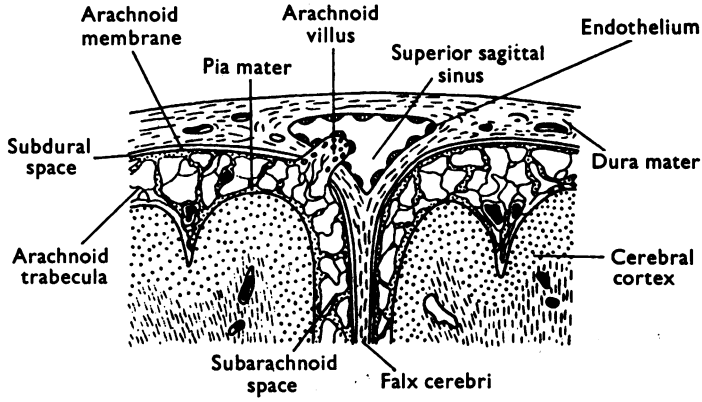
brain was able to obtain its nutriment from the blood, by way of the choroid plexuses, 'die Weg über den Liquor', the blood vessels of the brain being impermeable to most substrates. This view was later developed by von Monakow (1920) and Stern & Gautier (1921). They accepted the conccept of a barrier between blood and the nervous tissue, but, instead of viewing this as a restraint that slowed the passage of solutes from the blood vessels of the brain, they regarded it as an absolute barrier. There is no need to emphasize the intellectual inadequacy of the Stern–Gautier concept that attributes no nutritive role to the highly efficient vascular circulation of the brain parenchyma, and assign this completely to the limited vascular regions comprising the choroid plexuses, whose blood flow must represent only a small fraction of the total.

Blood–c.s.f.–brain. Let us now pass over the period between the qualitative studies of Goldmann, and the semi-quantitative studies of Stern & Gautier, and consider the ideas that have followed from quantitative studies carried out in many laboratories over the past 20 years or more. First we must examine the relations between the blood, c.s.f. and brain tissue, since these represent three main interrelated compartments, so that the discussion of the relations between blood and brain without at the same time considering the relations between c.s.f. and brain, and blood and c.s.f., is equivalent to studying the relations between pressure and volume of a gas without attempting to control its temperature. Text-fig. 1 shows the general relations of the c.s.f. and brain; the c.s.f. is contained within the ventricles where it is formed continuously by the choroid plexuses. It passes out of the ventricles into the subarachnoid spaces, i.e. the spaces between the pia, which invests the brain-surface closely, and the arachnoid mater, which invests the inner surface of the dura. Thus the irregularities of the brain's surfaces are bridged over by arachnoid to produce spaces of varying depth; in certain regions, notably the cisterna cerebellomedullaris (cisterna magna), the space is exceptionally large, and this constitutes a useful sampling locus for studies on experimental animals. A fluid formed continuously must be drained away; and this process takes place into the venous system, namely the large sinuses buried in the dura mater. At localized sites the dura is penetrated by arachnoid tissue which projects into the lumen of the vein or sinus, as indicated schematically in Text-fig. 2 and factually in Pl. 1. Controversy still exists as to the ultrastructure of the openings from the subarachnoid space into the lumen of the sinus (Shabo & Maxwell, 1968*a*, *b*; Alksne & Lovings, 1972; Tripathi & Tripathi, 1974), but the physiological evidence is unequivocal in demanding that the openings be capable of permitting a relatively unrestricted flow of fluid and solutes of large molecular weight such as sucrose, inulin, and plasma proteins (Davson, Hollingsworth & Segal, 1970; Davson,

Domer & Hollingsworth, 1973). Thus the openings may well be of microscopical size, but certainly of diameter greater than 100 Å. The essential point to bear in mind is that the way out for solutes in the c.s.f. is unrestricted, so that the fluid could act as a lymphatic system, providing a mechanism for clearance of substances that had found their way into the fluid either from the brain tissue directly as in cellular break-down, escape of transmitters, etc., or from the blood stream less directly.

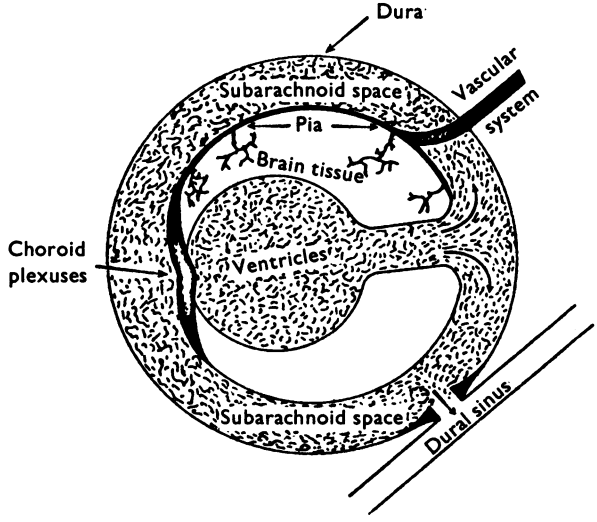


Text-fig. 1. Illustrating the ventricles and subarachnoid spaces, etc. A.G., arachnoid granulations; A.S., aqueduct of Sylvius; C.C.M., cisterna cerebello-medullaris; C.I., cisterna interpeduncularis; C.P.L.V., choroid plexus lateral ventricle; C.S., cisterna superior; D., dura mater; F.L., foramen of Luschka; F.M., foramen of Magendie; G.C.V., great cerebral vein; I.F., intraventricular foramen (Monro.); S.A.S., subarachnoid space; S.C.V., superior cerebral vein; S.S.S., superior sagittal sinus (after Netter, 1953).



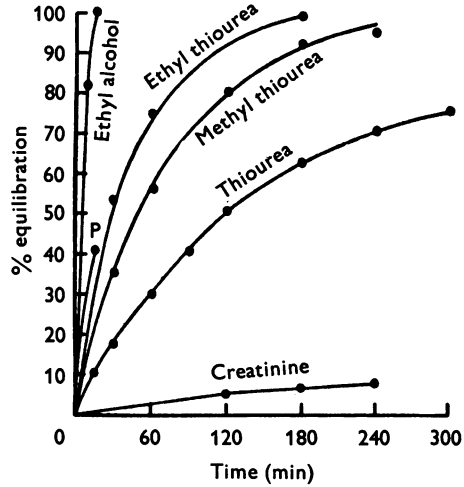
Text-fig. 2. Illustrating Weed's concept of the arachnoid villus as an out-pouching of arachnoid tissue into the dural sinus.

The relations between the three compartments (blood, brain and c.s.f.) are illustrated schematically in Text-fig. 3, where the ventricular system has been represented as a single cavity buried within the nervous tissue; it opens into the subarachnoid system surrounding the nervous tissue. Blood comes into relation with the compartments (*a*) in the choroid plexuses, (*b*) in the capillaries of the nervous parenchyma, and (*c*) in the dural sinuses.



Text-fig. 3. Diagram illustrating relations between cerebrospinal fluid and brain tissue (Davson, 1963).

Choroid plexuses. These are outpouchings of the vascular system into the ventricle, covered with ependyma which has become morphologically and functionally differentiated from its neighbouring regions, and is called the choroidal epithelium. Through active transport of salts associated with movement of water, the c.s.f. is secreted at these loci, an initial filtration from the blood capillaries into the stroma of the plexus constituting the basic solution from which the specialized fluid is made up. Passage of materials from the blood into the c.s.f. involves passage into this primary filtrate into the interstitium of the choroid plexus, and Goldmann's early studies indicated that there was no restraint on the passage of trypan blue out of the capillaries although passage onwards into the c.s.f. was restrained.

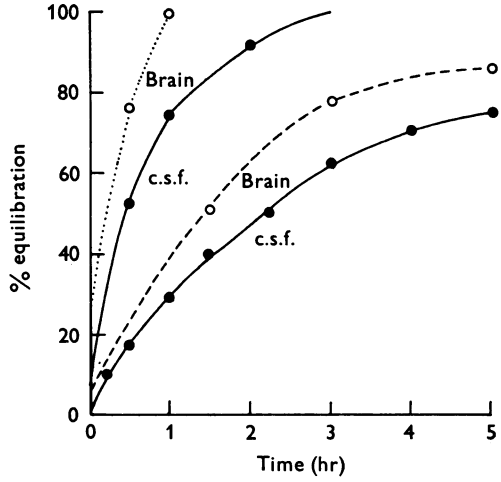


Text-fig. 4. The blood–cerebrospinal fluid barrier. A steady level of the solute (= 100) was maintained in the blood plasma, and at intervals the animal was anaesthetized and its cerebrospinal fluid removed for analysis (Davson, 1955).

Blood–c.s.f. barrier. Subsequent work has left little doubt as to the high permeability of the choroidal capillaries to large molecules, such as iodinated serum albumin (RISA), whilst passage from the interstitium into the c.s.f. is restrained, showing a high degree of selectivity that has given rise to the concept of the *blood–c.s.f. barrier.* The cells of the epithelial layer are sealed laterally to form 'tight junctions' (Burgess & Segal, 1970), and it is this morphological feature that constitutes the barrier to passage of dissolved material from blood to c.s.f. A quantitative example is provided by Text-fig. 4; this and a number of other studies indicate that passage is governed primarily by lipid solubility although, as we shall see, other factors are operative.

Exchanges between c.s.f. and brain. If we refer back to Text-fig. 3 we shall see that a foreign substance, injected into the blood, can pass, at any rate theoretically, out of the blood capillaries of the parenchyma into the extracellular space of the brain and thence into the c.s.f. as well as into the adjacent cells of the brain. Alternatively, of course, if we accept the Stern–Gautier hypothesis, passage into the brain could be secondary to the passage into the c.s.f., provided the ependymal and pial linings of the cavities containing the c.s.f. were sufficiently permeable.

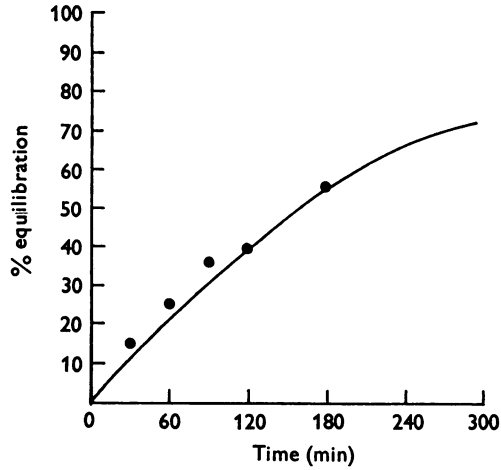
These linings are, indeed, highly permeable structures, lacking the occlusive tight junctions between adjacent cells characteristic of the choroidal epithelium. A variety of studies, dating from the previously quoted qualitative ones of Biedl & Kraus (1898), Lewandowsky (1900) and Goldman (1913), suggest an absence of selectivity of these membranes, and more accurate quantitative work, involving the controlled perfusion of the ventricles with solutions of known composition, has indicated that substances such as sucrose or inulin, which pass across the choroidal epithelium only with difficulty, pass readily into the adjacent brain parenchyma. Thus, functionally, the 'Weg über den Liquor' is practicable, but quantitative experiments indicate that it is unimportant as a means of supplying solutes to the brain and, in fact, that the c.s.f. acts rather as a 'sink' to the brain, receiving solute that has entered the brain from the blood capillaries and diffused across the ependyma. Thus Text-fig. 5 shows simultaneous measurements of uptake of two solutes from blood into brain tissue and



Text-fig. 5. The blood–cerebrospinal fluid and blood–brain barriers compared. Procedure was similar to that described in legend for Text-fig. 4, but brain was also analysed after homogenization. Upper curves: ethyl thiourea. Lower curves: thiourea (Davson, Kleeman & Levi, 1963).

c.s.f.; it will be seen that the concentration in the tissue water is consistently higher than in the c.s.f. throughout the approach to equilibrium, so that if there is a 'Weg über den Liquor' it is usually, if not invariably, in the opposite direction to that envisaged by von Monakow and Stern & Gautier.

The concept of the 'Weg über den Liquor', whichever way we consider it, is of the utmost value in underlining the intimate diffusional relations between c.s.f. and brain; thus to consider the curves of Text-fig. 4, the uptake into the c.s.f. must be compounded of a component of blood–c.s.f. barrier, in the sense of a passage across the choroid plexuses, and a component of brain–c.s.f. exchange, so that the treatment of the blood–c.s.f. system as a simple two-compartment system is theoretically incorrect, although mathematically it may well conform to such simple

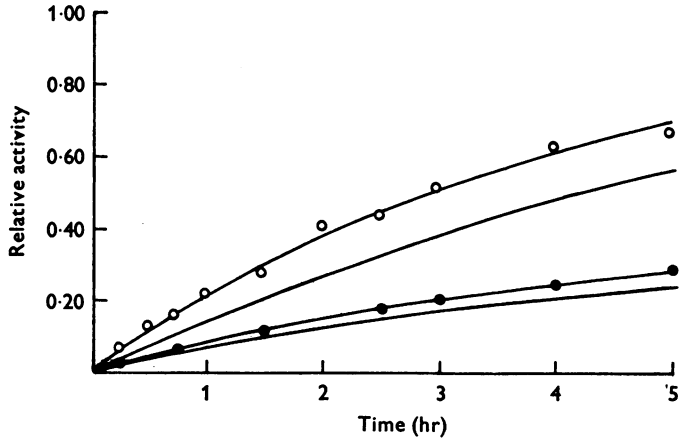


Text-fig. 6. Blood–cerebrospinal fluid and blood–brain barriers to ^{24}Na. Procedure was similar to that described in legend for Text-fig. 5. The continuous curve represents penetration into the cerebrospinal fluid and the dots represent penetration into the extracellular space (computed as the chloride space) of the brain (Davson, 1955).

kinetics. This is revealed by two studies on exchange of ^{24}Na between blood, on the one hand, and c.s.f. and brain extracellular fluid on the other. Text-fig. 6 is derived from an early study (Davson, 1955) in which uptake into brain extracellular fluid – taken as the Cl space – and c.s.f. were measured in the same animals. The curve indicates passage into c.s.f. and may be described by a simple logarithmic function obtained by integrating the simple two-compartment equation:

$$dC/dt = K(C_{plasma} - C_{c.s.f.}).$$

The points on the curve indicate the uptake into the brain, and it will be seen that there is a remarkable approximate identity in the two courses of uptake. This implies that the kinetics of uptake from blood to brain on the one hand, and from blood to c.s.f. on the other, are matched, so that during the approach to equilibrium there is probably little exchange between the c.s.f. and the brain. Text-fig. 7 from Davson & Welch (1971)



Text-fig. 7. Blood–cerebrospinal fluid and blood–brain barriers to ^{24}Na. Similar type of experiment to that described in Text-fig. 6. Open circles represent activities in cerebrospinal fluid, and filled circles activities in brain-water. The curves have been calculated from equations that take into account exchanges between cerebrospinal fluid and brain extracellular fluid and between the extracellular and intracellular fluids. In addition, lines are superimposed showing how a decrease in rate of secretion of cerebrospinal fluid by 50% would affect the kinetics (Davson & Welch, 1971).

indicates the results of a more elaborate experimental study in which the same parameters were measured, but the processes were analysed mathematically in considerable depth, exchanges between blood, c.s.f., brain extracellular fluid, and brain cells all being included. The experimental points fit well to the computerized solution to the equations describing uptake into the two compartments, but it turns out that the error involved in treating each pair of compartments separately, as implied by Text-fig. 6, is in this case quite small. This is probably a unique situation, however, and the exchanges of K^+ or thiourea between the compartments are more complex and demand the more exact mathematical treatment.

Sink-action of c.s.f. The importance of exchanges between c.s.f. and brain becomes very evident when passage of slowly penetrating substances, such as creatinine, sucrose, iodide, *p*-aminohippurate, etc., from blood into the brain is being measured. The striking feature of this penetra-

tion is that it apparently never reaches 'completion', in the sense that, at an 'infinite time' after establishing a steady concentration in the plasma, the concentration in the extracellular fluid is considerably less than in the plasma. On thermodynamic grounds we should expect the two concentrations to become equal in the absence of some disturbing force; and the disturbing force is, apparently, the diffusion of the solute from the extracellular fluid of the brain into the c.s.f. Thus the concentration in the c.s.f. is, in fact, lower than in the extracellular fluid, owing to the blood c.s.f. barrier which exerts a strong restraint on passage of sucrose, say, across the choroidal epithelium. The c.s.f. is a flowing system, and we have seen that there is no constraint on escape of the fluid with its sucrose into the dural sinus blood through the arachnoid villi. Thus penetration into the c.s.f. will follow an equation of the form:

$$dC/dt = K_{in} C_{plasma} - K_{flow} C_{c.s.f.}$$

At the steady state, when $dC/dt = 0$, we have

$$C_{c.s.f.}/C_{plasma} = K_{in}/K_{flow}$$

Thus if K_{in} is small compared with the flow constant, a steady state is reached with the concentration in the c.s.f. less than in the plasma, and in fact steady-state ratios of the order of 0·1–0·01 are obtained with slowly penetrating substances. Consequently, if the exchange between brain extracellular fluid and c.s.f. is unrestricted, the c.s.f. must act as a sink preventing the build-up of the concentration of the slowly penetrating solute, such as sucrose, in the extracellular fluid of the brain. Thus we do not have to invoke active processes keeping such solutes out of the brain, but merely a passive restraint coupled with a flowing system, the c.s.f.

Facilitated transfer and active transport. Studies on the permeability of cell membranes have indicated that the kinetics of transport are often anomalous, the calculated permeability coefficient being dependent on the concentration of the permeating solute. The transport is analogous with that which would be expected were the molecules or ions required to attach themselves to a 'carrier' with a special affinity for the solute; as the concentration of the solute increased the proportion of available sites on the carrier to the number of molecules awaiting transport would decrease, and so the permeability would show 'saturation kinetics'. Typical substances involved in this 'carrier-mediated transport' are sugars, such as glucose and mannose; these are highly water-soluble and, unless some carrier mechanism were available, they would be virtually excluded from c.s.f. and brain; but in fact it was early established, using chemical techniques, that glucose penetrated relatively rapidly into c.s.f. (Davson, 1955). A variety of studies on monosaccharides have shown that uptake

into the c.s.f. and brain exhibits saturation kinetics. Especially valuable for this type of study has been the technique of 'indicator dilution' developed by Crone (1965) and later by Yudilevich & Rose (1971). Here the monosaccharide is infused into the internal carotid artery together with a solute (the indicator) that does not cross the blood–brain barrier appreciably, e.g. inulin or Evans blue, and the change in concentrations of the monosaccharide and indicator as blood emerges from the torcular vein permits an estimate of 'extraction'. With this technique it has been shown that the transport of a given labelled sugar, present in only tracer concentrations, is inhibited by addition of unlabelled sugar to the injection solution (self-inhibition) and when different sugars are compared, striking differences in rate of transport are found. When mixtures of sugars are employed, there is mutual competition for transport.

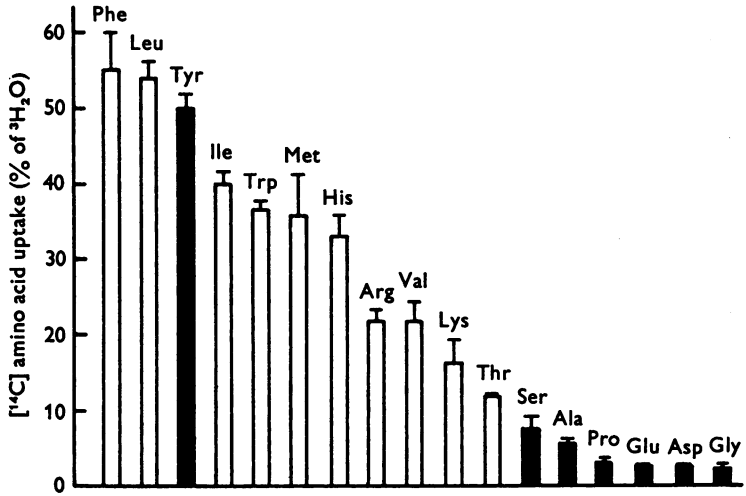
Oldendorf (1971) has used an ingenious method for studying uptake of solutes into the rat's brain. Labelled solutes, such as sugars, are incorporated in an isotonic saline solution and injected into the carotid artery, and after some 15 sec the animal is decapitated and the amount of radioactivity in the brain estimated. In order to be able to compare one experiment with another, a standard of reference is included in each injection, and this is a known activity of tritiated water. This penetrates brain very rapidly, coming into approximate equilibrium during the first passage of the blood, so that if the extraction of the amino acid is compared with the extraction of the tritiated water, a ratio is obtained which makes allowance for different rates of blood flow, etc. The results on a series of hexoses are shown in Table 1; the very low uptakes of the unnatural D-fructose and L-glucose indicate the stereospecificity of the carrier. When unlabelled carrier was added to the injecting fluid, uptake was suppressed; thus the index for D-glucose was decreased from 33, when the injected concentration was 0·42 mM, to 9·4 at 80 mM. The results of a study on a series of

TABLE 1. Penetration of some labelled hexoses into the rat's brain. The uptake is expressed as a % uptake of ^3H_2O incorporated into the intra-arterial injection fluid (Oldendorf, 1971)

	Injected concn. (mM)	Brain uptake index
^3HO reference		100
2-Deoxy-D-glucose	0·02	46 ± 4
D-Glucose	0·42	33 ± 3
3-*O*-methyl-D-glucose	0·06	29 ± 2·5
D-Mannose	0·05	21 ± 1·3
D-Galactose	0·21	14·4 ± 1·6
D-Fructose	0·43	1·75 ± 0·32
L-Glucose	0·42	1·63 ± 0·46

Brain uptake index values are means ± S.E. For each mean $n = 3$.

amino acids are shown in Text-fig. 8; as Baños, Daniel, Moorhouse & Pratt (1973, 1975) had found in a less detailed study, essential amino acids penetrate more rapidly than inessential ones. This is especially striking where amino acids that are probably transmitters in the central nervous system, e.g. glycine, are concerned; their penetration into the brain is greatly restricted. The penetration is of the carrier-mediated type, in the sense



Text-fig. 8. Relative uptakes of amino acids by rat brain when a single bolus of the labelled amino acid is injected into the carotid artery, together with ^3H_2O. In the rat, tyrosine is probably an essential amino acid (Oldendorf, 1971). Open blocks, essential amino acids; filled blocks, non-essential amino acids.

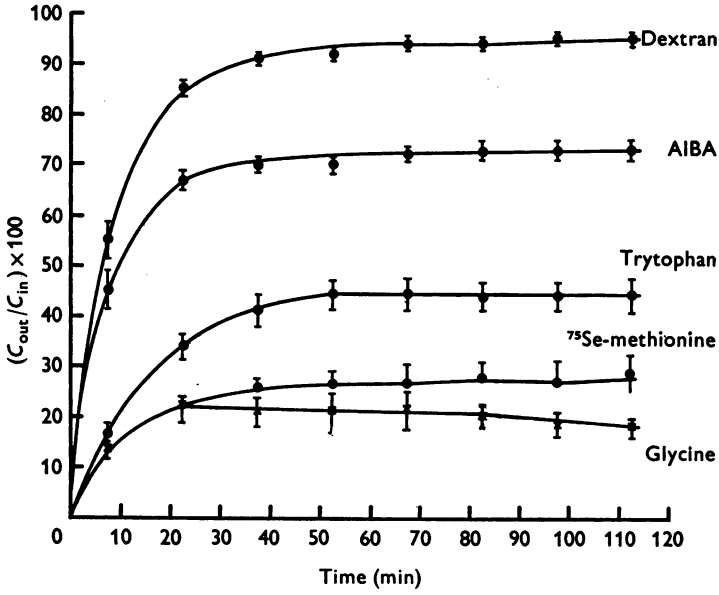
that extraction from the blood of the labelled amino acid is reduced when additional, non-radioactive, amino acid is added. Moreover, as found in other systems, different amino acids compete with each other for transport.

Physiological significance of facilitated transport. The role of this facilitated, carrier-mediated, transport is at first sight obvious; a barrier that operates on the basis of lipid solubility would be a physiological nuisance where necessary lipid-insoluble metabolites are concerned, and the cellular membranes have been modified to permit transport of the necessary metabolites. The position is a little more complex, however, since there is strong evidence that, although transport is facilitated inwards, in the sense that its rate is greatly in excess of that to be anticipated of molecules of similar structure, the transport outwards, i.e. from c.s.f. to blood, is facilitated to an even greater extent, so that there is a net tendency for the

solutes to move against gradients of concentration or, where ions are concerned, of electrochemical potential. Thus the steady-state concentration of glucose in the c.s.f. is about 60 % of that in the plasma of all species examined (Davson, 1956). It could be argued that transport inwards and outwards was governed by passive forces only, i.e. by the concentration gradients, its rate both ways being facilitated by a carrier mechanism, whilst the low c.s.f. concentration would be due to the metabolism of the neurones and glial cells in the adjacent tissue. This would imply, of course, that the transport of glucose out of the blood vessels of the brain was sufficiently restricted that the concentration in the extracellular fluid was about 60 % of the plasma level, owing to consumption by the cells. This is not an unreasonable proposition since consumption of glucose within cells is considered to be so rapid that the internal concentration is usually very small indeed, the rate of utilization of glucose being governed, essentially, by the rate at which glucose enters the cells. However, Csáky & Rigor (1968) have shown that the isolated choroid plexus can accumulate the glucose analogue, 3-methylglucose. Earlier studies in which glucose had been perfused through the ventricles (Bradbury & Davson, 1964) certainly indicated that removal was relatively rapid, and had carrier-mediated characteristics, but the critical experiment *in vivo*, to see whether the transport outwards continues in the face of an uphill gradient, has not so far been performed. The situation with amino acids is very similar; their steady-state concentrations in c.s.f. are usually very considerably lower than in plasma (Bito, Davson, Levin, Murray & Snider 1966*b*; Plum, 1974). Once again, it could be argued that the low c.s.f. concentration was due to the involvement of the amino acids in protein turn-over in the brain, so that the amino acids, diffusing passively into the tissue from blood in the brain capillaries, would be taken up rapidly into the cells, leaving a low steady-state concentration in the extracellular fluid. This would act as a sink for the amino acids passing into the c.s.f. from the blood, and lead to a low steady-state level in both c.s.f. and extracellular fluid.

When amino acids are perfused through the ventricles in the typical ventriculo-cisternal perfusion experiment, the steady-state level reached varies with the amino acid, thus as Text-fig. 9 shows, glycine, selenomethionine and tryptophan reach a low level, indicative of high clearance from the perfusion fluid, whilst the steady-state level for α-aminoisobutyric acid (AIBA) is much higher, indicating a smaller clearance. The transport outwards exhibits carrier features in so far as addition of inactive amino acid to the labelled amino acid suppresses clearance, as shown by the rise in steady-state concentration (Text-fig. 10). This could be a reflexion of activity in the choroid plexuses, but of course it could also reflect activity in the adjacent brain tissue. Thus studies on isolated tissue

(Lajtha, 1968; Neame, 1968) indicate that the brain can accumulate amino acids to varying extents, and well above the level in the outside medium. Such an accumulation exhibits carrier-type kinetics, so that addition of inactive amino acid suppresses accumulation of the labelled material. Thus, in the ventriculo-cisternal perfusion experiment, addition of inactive amino acid would reduce losses to the adjacent brain cells, and



Text-fig. 9. Ventriculo-cisternal perfusion of rabbit with artificial cerebrospinal fluid containing an amino acid together with a coloured dextran of high molecular weight. The lower the steady-state level, the more actively is the amino acid removed from the perfusion fluid. The steady-state level of the dextran is determined by the secretion of new cerebrospinal fluid, since escape by way of the choroid plexuses, or into the brain, is negligible (H. Davson and J. R. Hollingsworth, unpublished).

this might account completely for the effect on the level in the perfusion fluid. By analysing the brain it is possible to determine whether addition of the inactive material suppresses accumulation of labelled material in this tissue. In fact, such a suppression was found by Lorenzo & Snodgrass (1972).

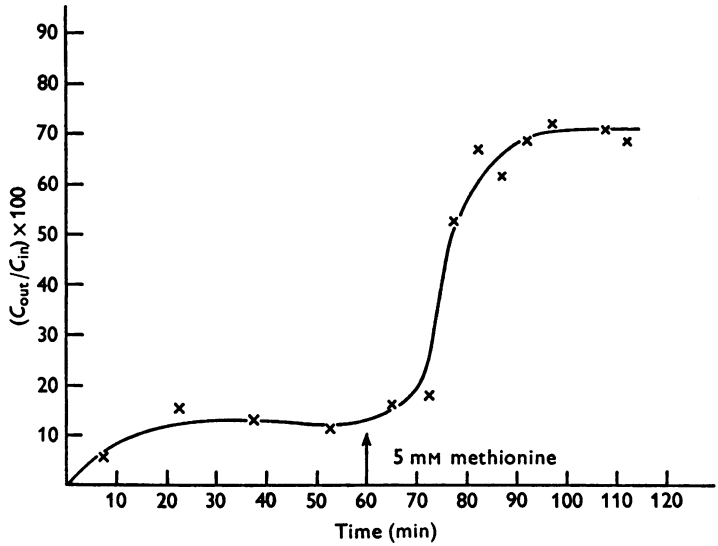
However, in experiments on AIBA carried out in my laboratory by Dr Gordon McComb it was easy to calculate that the suppression of uptake by the brain was inadequate to account quantitatively for the suppression of clearance from the ventriculo-cisternal perfusion fluid, so that the choroid plexuses undoubtedly share in the carrier-mediated type of transport.

As with glucose, we may ask whether the transport of amino acids from blood into and out of the c.s.f. is passive – carrier-mediated, but governed by gradients of concentration – or whether the low levels normally found in the c.s.f. by comparison with those in the blood plasma are the result of an active transport directed from c.s.f. to blood. In this case the critical experiments have been carried out; [^{75}Se]selenomethionine was used as a convenient analogue of methionine, emitting a gamma radiation. Text-fig. 11 shows the radioactivities of the inflowing and outflowing fluid perfusing the rabbit's ventricles; the activity of the inflowing fluid (C_{in}) is constant and represented by the horizontal straight line. The activity in the outflowing fluid (C_{out}) reaches a steady-state level less than one third of that in the inflowing fluid. At the arrow, an intracarotid infusion of radioactive selenomethionine was begun, and the radioactivities in the torcular blood are indicated in the uppermost curve. These are from 20 to 15-times the activities in the perfusion fluid as it emerges from the cisterna magna, so that if clearance from the perfusion fluid had depended only on passive (but carrier-mediated) diffusion into the choroid plexuses, then clearance should have ceased soon after the beginning of the intra-carotid infusion, the time for an effect on the value of C_{out} being governed by the dead-space of the system. In fact there is only a slight drift upwards, suggesting a small increase in influx, but the net efflux of labelled selenomethionine remained in the direction of a movement up a gradient of activity. That the time allowed for an effect of the raised blood-level was adequate has been shown by other experiments in which clearance of selenomethionine was reduced by switching from a carrier-free to a carrier-containing perfusion fluid (Text-fig. 10); the ratio: C_{out}/C_{in} starts to rise within 10 min of establishing the altered conditions and a new steady-state is reached within 20 min.

It might be argued that the low steady-state level during the perfusion was due to uptake by the brain cells, rather than to any active process in the choroid plexuses, in which case the level in the blood would not necessarily be relevant until the brain had reached its own steady state, which might require many hours. However, by analysis of the brain it was shown that the total contribution of this tissue to the loss from the perfusion fluid was 38 %, so that the major loss was, indeed, through the choroid plexuses and thus represented a movement against a gradient of chemical potential, i.e. it was active transport.

Active transport in the brain. The close relations between c.s.f. and brain tissue, both spatial and diffusional, have led to the thesis that the extracellular fluid of brain is essentially equivalent to c.s.f. in so far as their chemical compositions are concerned (Davson, 1958), so that, because the c.s.f. can be sampled, whilst estimates of concentrations in the brain extra-

cellular fluid are of their nature indirect, I have felt justified in including a discussion of the blood-c.s.f. relations into this discussion of blood–brain relations; in fact, to understand the complete picture such a discussion is absolutely necessary. The studies so far enumerated have shown that the c.s.f. has a chemical composition different from that of a protein-free filtrate of plasma, the concentrations of glucose and most amino acids being very much less in the c.s.f. A similar situation, but not so obvious,

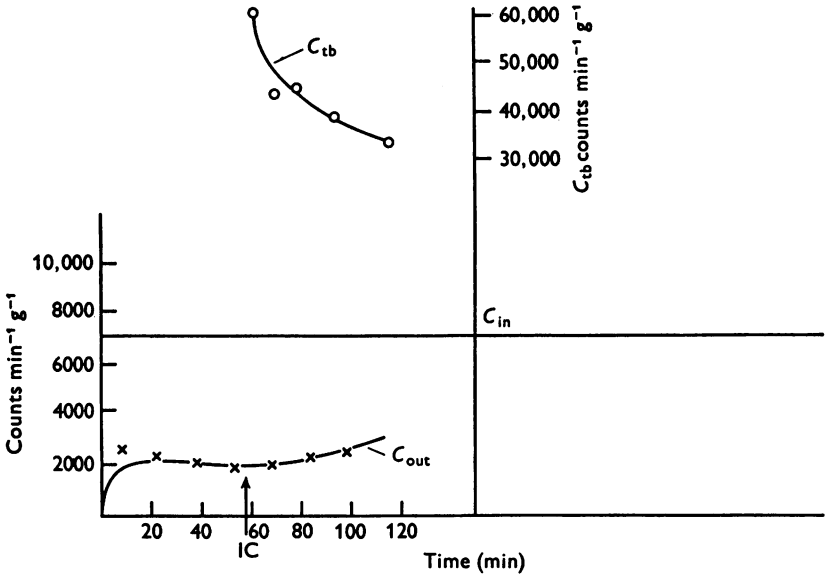


Text-fig. 10. Ventriculo-cisternal perfusion with artificial cerebrospinal fluid containing [^{75}Se]selenomethionine. At the arrow the perfusion fluid was changed to one containing identical activity of [^{75}Se]selenomethionine but in addition 5 mM methionine (H. Davson and J. R. Hollingsworth, unpublished).

TABLE 2. Concentrations of various solutes (m-equiv/kg H_2O) in plasma and lumbar cerebrospinal fluid of human subjects. $R_{c.s.f.}$ is the ratio of cerebrospinal fluid to plasma concentrations (Davson, 1967)

Substance	Plasma	C.s.f.	$R_{c.s.f.}$
Na	150	147	0·98
K	4·63	2·86	0·615
Mg	1·61	2·23	1·39
Ca	4·70	2·28	0·49
Cl	99	113	1·14
HCO_3	26·8	23·3	0·87
Br	2·45	0·90	0·37
Inorg. P (mg/100 ml.)	4·70	3·40	0·725
Osmolality	289	289	1·0
pH	7·397	7·307	—
P_{CO_2} (mmHg)	41·1	50·5	

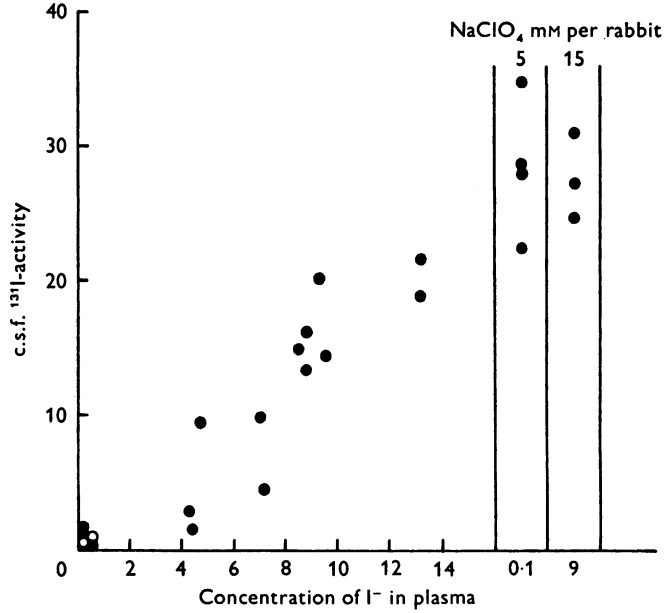
prevails where ions are concerned (Table 2), so that the concentration of K^+ is some 2·9 m-equiv/l. compared with a plasma value in the region of 4·6 m-equiv/l.; the concentration of Mg is 2·23 compared with 1·61 for plasma, and so on. We have seen that active transport mechanisms probably govern the concentrations of glucose and amino acids in the c.s.f.,



Text-fig. 11. Ventriculo-cisternal perfusion with artificial cerebrospinal fluid containing [^{75}Se]selenomethionine. At the arrow an intra-carotid infusion of saline containing [^{75}Se]selenomethionine was begun. Ordinate to the left indicates radioactivity of the perfusion fluid, that of the inflowing fluid being indicated by the horizontal line (C_{in}). The crosses indicate activity of the out-flowing fluid (C_{out}). Ordinates at the right indicate activity in the blood-plasma taken from the torcular vein (C_{tb}). Note that in spite of the steep gradient of radioactivity between C_{in} and C_{tb}, the activity in the fluid leaving the ventricles, C_{out}, remains well below that of the inflowing fluid (C_{in}) (H. Davson and J. R. Hollingsworth, unpublished).

and the same is unequivocally true of such ions as have been studied in detail, such as K^+, Cl^- and Na^+. Very striking is the active transport of the bromide, iodide and thiocyanate ions; because perchlorate is a specific inhibitor of this type of transport in several systems, e.g. the thyroid, the factors involved in this active transport have been analysed in some detail. Text-fig. 11 shows the typical ventriculo-cisternal perfusion curve; at the arrow, an intraperitoneal injection of perchlorate was given, and the suppression of outflux results in a rapid increase in level of the radioactivity in the outflowing fluid. When radioactive I^- is infused into the

blood, the concentrations built up in the c.s.f. and brain are very low; the low levels in the c.s.f. are clearly the consequence of the active flux outwards, since treatment of the animal with inactive I^- or perchlorate leads to a much more rapid and extensive passage of ^{131}I from blood into this fluid (Text-fig. 12); the same is true for the brain, so that it might be argued that the low uptake of carrier-free ^{131}I from blood into brain was



Text-fig. 12. Effects of plasma I^- and perchlorate on the cerebrospinal fluid/plasma concentration ratio achieved after 2 hr (open circles) and 6 hr (filled circles) of I.V. infusion of ^{131}I. Ordinate, ^{131}I-activity in cerebrospinal fluid as percentage of that in a plasma dialysate. Abscissa, concentration of free I^- in plasma (m-mole/kg H_2O). Points between vertical lines correspond to animals in which perchlorate was administered (Bito, Bradbury & Davson, 1966*a*).

the consequence of an active transport of the ion from brain extracellular fluid into the blood comparable with that from c.s.f. into the blood. In this latter case the choroid plexuses are responsible for the active transport, whereas in the brain the active transport would be 'across the blood–brain barrier', and the active cells might be the capillary endothelial cells or the astrocytic layer of glial cell-processes that envelops the brain capillaries (Text-fig. 13). To prove an active transport across the blood–brain barrier was not easy because of the 'sink action' of the c.s.f.; thus the low level of activity of ^{131}I in the c.s.f. would ensure a low level of activity in the brain, quite independently of any active transport by the brain

capillaries or glial cells. The results of several studies (Bito, Bradbury & Davson, 1966*a*; Ahmed & Van Harreveld, 1969; Coben & Smith, 1969; Davson & Hollingsworth, 1973) have led to the conclusion that the iodide-ion is, indeed, actively transported out of the extracellular fluid into the blood, in parallel with an active transport of iodide from the c.s.f. Teleologically we might say that these two processes have developed to ensure that the concentrations of bromide, iodide and thiocyanate are unlikely ever to reach values that would be toxic to the central neurones.

Text-fig. 13. The astrocytic covering of a brain capillary (drawing after Wolff, 1963).

Prostaglandins. These local hormones are produced within the brain, where they probably act as primitive transmitters; the brain has no chemical inactivating mechanism for these substances once they have served their function, in contrast with the situation with the amines where there is either hydrolysis (as with acetylcholine) or re-uptake at the nerve terminals as with catecholamine and amino acid transmitters. So effective are these latter mechanisms that it seems unlikely that the active trans-

port of these transmitters from the c.s.f. serves an important function in inactivation; rather the active transport is a reflexion of the barrier function, restraining passage of the transmitters from the blood into the brain. In the absence of local re-uptake or metabolic inactivation mechanisms, the prostaglandins must be removed by the blood. According to Bito's recent studies, the prostaglandins that are active in the brain, e.g. $F_{2\alpha}$, have a very low passive permeability through cells, so that unless an active, uphill, transport mechanism has developed, as in the ciliary body of the eye (Bito & Salvador, 1972) or across the rabbit vagina (Bito & Spellane, 1974) diffusion through epithelium-like membranes will be very restricted. That the transport of $PGF_{2\alpha}$ out of the c.s.f. has carrier-mediated characteristics is shown by Text-fig. 14 (Bito & Davson, 1974) where the ratio C_{out}/C_{in} for a ventriculocisternal perfusion of carrier-free $PGF_{2\alpha}$ has been plotted against time. A steady-state level of activity is reached between 60 and 90 min, with the outgoing fluid having an activity some 55 % of the inflowing fluid. With the large-molecular-weight Blue dextran, which does not escape appreciably into the blood or adjacent brain, the steady-state level is 87 %, the fall in concentration compared with that in the inflowing fluid being due to secretion of new fluid. At the arrow, a new perfusion fluid containing the same activity of $[^3H]PGF_{2\alpha}$ but, in addition, some non-radioactive $PGF_{2\alpha}$ (500 μg/ml.) was perfused; the rise in steady-state level indicates suppression of clearance by saturation of a carrier-mediated process. It is likely that this represents more than facilitated transport, i.e. it indicates a transport against concentration gradients; at any rate the isolated choroid plexus can accumulate PG's (Bito, Davson & Salvador, 1976), a process that is inhibited by carrier concentrations of the prostaglandins.

This active removal of PGs from the c.s.f. would permit the fluid to act as a sink for PGs liberated during nervous activity in the brain; and if the analogy with other active processes in the brain–c.s.f. system extends to the PGs, an additional active transport across the blood–brain barrier would serve to remove the PGs locally. This finding gives meaning to the well established active transport of a group of organic acids from the c.s.f. Thus it was early remarked that the rate of removal of *p*-aminohippurate from the c.s.f., when injected into the subarachnoid space, was very rapid by comparison with that of, say, sucrose, although both penetrated equally slowly from the blood (Davson, 1956); and by applying the technique of ventriculo-cisternal perfusion to the problem, Pappenheimer, Heisey & Jordan (1961) showed that *p*-aminohippurate belonged to a group of substances, including phenolsulphonephthalein and Diodrast, which, as in the kidney proximal tubule, were actively transported. In the kidney, the process is from blood to tubule but in the choroid plexus it is

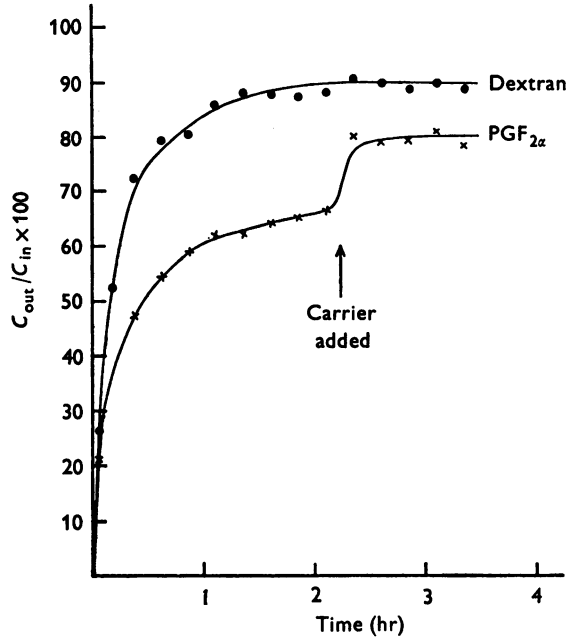
from c.s.f. to blood. In both instances, however, the active transport is directed to clearing the appropriate fluid (blood or c.s.f.) of an unwanted type of compound. So far as the kidney is concerned the significance of this transport mechanism is probably connected with the elimination of urates and the large variety of glucuronic acid conjugates that appear in the blood. The significance of the system in the c.s.f. (and in the eye) has until now been a puzzle, but its value in removing prostaglandins from the brain is now obvious. Just as with the kidney transport mechanism, so with the transport of PGs across the choroid plexus, the substances that are actively transported fall into the same categories and their transport is inhibited by the same substances, e.g. bromcresol green and probenecid.

Site of the blood–brain barrier. Because a characteristic feature of the brain capillary is its close investment by glial cells, the low permeability of the blood–brain barrier has been attributed to this glial covering, but with the demonstration by Brightman (1965) that large electron-microscope tracers, such as ferritin, could pass between the gaps between the glial cell processes, a primary role of the capillary endothelium was postulated, although the failure to establish any morphological difference between the capillaries of, say, skeletal muscle and those of the brain, posed a puzzling problem which was only resolved when Reese & Karnovsky (1967) showed that, whereas the intercellular clefts of muscle capillaries allowed the passage of horseradish peroxidase (mol.wt. 40,000), this electron-microscope marker was unable to pass through the clefts in brain capillaries. Thus the capillaries of brain are different from those of muscle, and it could be that the formation of tight junctions, sealing the intercellular clefts, constituted the morphological feature that lay at the basis of the restricted permeability. Can we also regard the endothelial cell as the analogue of the choroidal epithelial cell, actively transporting iodide, amino acids, sugars, prostaglandins, etc., from extracellular fluid into the blood? It has been argued that the paucity of mitochondria in the endothelial cells of the capillary would rule out such a function, requiring as it does a supply of metabolic energy. In this event we must invoke the glial cells, with their 'foot-processes' resting on the capillary endothelial cells. They might transport solutes from the extracellular fluid into the clefts between them and the endothelial cells; the local high concentrations built up might allow passive diffusion into the lumen of the capillary and thus achieve an effective uphill transport from extracellular fluid to blood. The objection that active transport would require a higher density of mitochondria in the endothelial cells is not very cogent in view of the active transport exhibited by the mammalian erythrocyte, and by W. H. Oldendorf's (personal communication) finding that the density of mitochondria in capillary endothelial cells of the brain is significantly higher than in

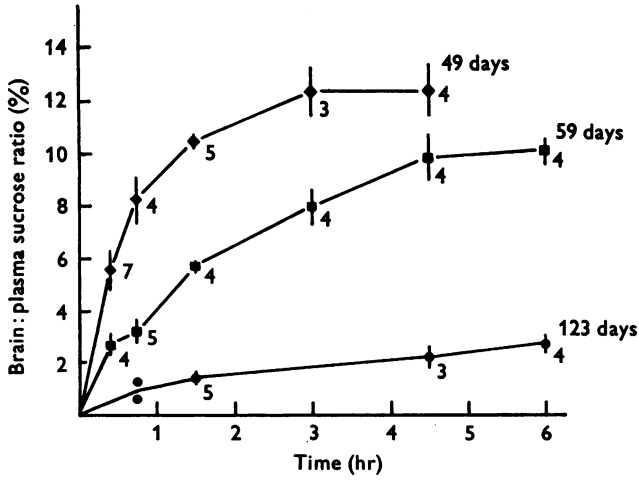
capillary endothelial cells of non-brain tissue. Thus, in the absence of direct evidence indicating an active transport role for the astrocytes associated with the blood vessels, it is simplest to attribute the special features of the blood–brain barrier, namely specificity for individual hexoses and amino acids including stereospecificity (Oldendorf, 1973), active transport of anions, and so on, to the capillary endothelial cells.

Ontogeny of the blood–brain barrier. It has been generally held that, except in those species such as the guinea-pig that are born at a high state of maturity, the blood–brain barrier at birth is immature, so that trypan blue or thiocyanate, which are virtually excluded from the adult brain when given intravenously, penetrate into the brain of new-born rabbits, kittens, etc. (Stern & Peyrot, 1927). In the late foetal stage the barrier, on this basis would be even less mature, so that Spatz (1934) attributed the frequent association of kernicterus with haemolytic and other forms of jaundice in the new born to the absence of a barrier to the bile pigments in the foetal brain. Spatz' attribution of kernicterus to an immature barrier is unsound, the defect being a failure to conjugate the highly lipid-soluble bilirubin which, in this unconjugated condition, can cross the blood–brain barrier and exert its toxic effects (Diamond & Schmid, 1966). As we might expect, the degree of maturity of the barrier in the new born does vary with the species, so that in the rat, for example, Ferguson & Woodbury (1969) found that, 4 days before birth, the barrier to sucrose and inulin is not high, so that when these substances were injected into the foetus, the concentration in the c.s.f. rose to about that in the plasma after 24 hr, whereas in the adult the corresponding concentration would be only 1 or 2 % of the plasma level. In sheep, on the other hand, the studies of Saunders and his colleagues (Evans, Reynolds, Reynolds, Saunders & Segal, 1974), carried out on foetuses at different stages of gestation with placentae intact at Caesarean operation, have shown that by 123 days, i.e. some days before parturition, the blood–brain barrier to sucrose is quantitatively similar to that of the new-born and adult animal; as Text-fig. 15 shows, at 49 days and 59 days penetration is both more rapid and more extensive, so that the final brain/plasma ratio achieved at 49 days is approximately equivalent to the sucrose space.

Role of the blood–brain barrier in homoeostasis. The feature of the blood–brain barrier with which we have been mostly concerned has been its ability to slow the exchanges between blood, on the one hand, and the c.s.f. and extracellular fluid of the brain on the other. Such a slowing might be regarded as a 'second line of defence' in the homoeostasis of the brain cells' environment, the homoeostatic mechanisms operating to maintain blood-plasma levels within a narrow range constituting the 'first line of defence'. This slowing of exchanges is made more effective by the operation



Text-fig. 14. Ventriculo-cisternal perfusion with artificial c.s.f. containing [^{3}H]$PGF_{2\alpha}$ and dextran of high molecular weight. At the arrow, the perfusion fluid was changed to one with identical radioactivity but with non-radioactive $PGF_{2\alpha}$ (500 μg/ml.) (Bito & Davson, 1974).



Text-fig. 15. Illustrating ontogenetic development of the blood–brain barrier in foetal sheep. A steady level of [^{14}C]sucrose was maintained in the blood and the brains were removed at definite times. Ordinate: c.p.m. per gram brain/c.p.m. per gram plasma-H_2O. Abscissa: time from beginning of infusion (Evans, Reynolds, Reynolds, Saunders & Segal, 1974).

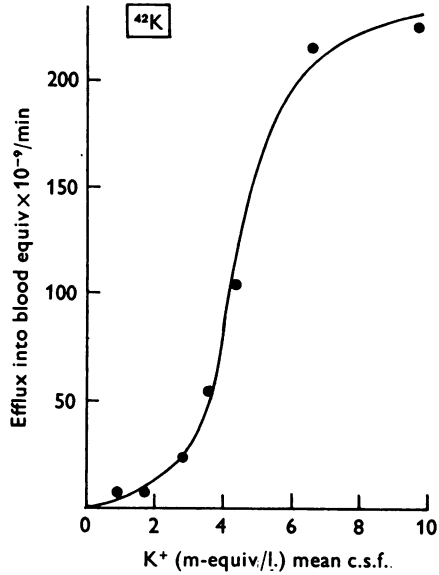
of active transport mechanisms that force certain solutes uphill from extracellular fluid (or c.s.f.) into the blood. To this extent our view of the barrier is rather a negative one, and is thus far too limited. However, we have seen that, where hexoses and amino acids are concerned, the behaviour of the barrier is equivocal; exchanges between blood and brain and c.s.f. are accelerated, in the sense that they take place much more rapidly than would be expected of molecules of comparable size and lipid solubility, but, on the other hand, the net tendency of the transport system is to accelerate the substances *out of* the brain and c.s.f., thus both influx and efflux are high but efflux is, apparently, higher than influx, leaving a steady-state situation with the c.s.f. and brain extracellular fluid levels less than in plasma.

A possible interpretation of this paradox is that the transport system is primarily concerned with homoeostasis, i.e. with the maintenance of a constant concentration of the various solutes in the extracellular fluid of the brain, and, of course, in the c.s.f. To meet the requirements of the system, fluxes of sugars and the essential amino acids must be high, but homoeostasis can perhaps be best achieved by maintaining a level different from that in the plasma, in the same way that body temperature is regulated, not at the average environmental temperature but at one considerably greater than this. Thus the homoeostasis of the brain-glucose level is maintained by a transport process that is adapted to maintain the concentration at some 35% below the plasma level. Specifically, this is achieved by a carrier-mediated system that becomes half saturated at about 5 mM or 90 mg/100 ml.; thus transport into the brain is governed by a process that tends to become saturated at plasma levels above the average, so that in severe hyperglycaemia the net transport is reduced below what would be expected on simple differential kinetics; by contrast, when the plasma levels fall in hypoglycaemia, the transport into the brain is favoured. According to the study by Pappenheimer & Setchell (1973), such a system is adequate to maintain glucose consumption by the brain over a fairly wide variation in plasma levels, although these authors account for the low level in the c.s.f. and brain extracellular fluid in terms of passive carrier-mediated transport without active efflux, i.e. in terms of consumption by the cells. In fact, however, the studies of Csáky suggest that, as well as a continuous consumption of glucose, tending to maintain the concentration low, an active transport out may well contribute. Similar situations may exist with respect to the amino acids, but the fact that some are transmitters makes broad generalizations less possible. The transmitters, such as glycine, can be synthesized within the brain so that the fluxes in either direction need not be high and, in fact, they are low and saturated at low concentrations. The active transport of glycine out of the

c.s.f. and brain is easily demonstrable, as we have seen. With amino acids required for brain protein synthesis – the essential amino acids – transport rates are generally much higher but, as with glucose, a net active outward transport seems to take place, contributing to the low steady-state levels of individual amino acids in plasma and brain extracellular space.

Where ions, such as Na^+, K^+, Cl^-, etc., are concerned, a utilization by the brain does not come into the question, so that the homoeostatic mechanisms are less complex. The best-studied ion in this respect is K; the steady-state level in c.s.f. is, as indicated earlier, some 2·9 m-equiv/l. compared with about 5 for the plasma. This level is maintained at approximately the same value in spite of large variations in the plasma level, sustained over long periods (Bekaert & Demeester, 1951, 1952; Bradbury & Kleeman, 1967). If the flux from plasma to c.s.f. were carrier-mediated, showing saturation at plasma levels corresponding to the normal c.s.f. level, namely 2·9 m-equiv/l., we might understand how raising the plasma level of K^+ failed to influence the level in the c.s.f. The studies of Bradbury & Kleeman (1967) and of Ames, Higashi & Nesbett (1965) suggested the existence of such a saturable influx mechanism, but that this, of itself, was inadequate to maintain homoeostasis of the c.s.f. level without an efflux mechanism from c.s.f. to plasma that responded in the opposite manner, showing *increased* efflux as the c.s.f. level rose. To demonstrate such a transport process is difficult, since, when the ventricles are perfused with an artificial c.s.f. containing ^{42}K, the losses of the isotope from the perfusion fluid are compounded of an efflux across the choroid plexuses into the blood (the efflux we are interested in) together with efflux into the adjacent brain-tissue; this latter component is high because the tissue has a high concentration of K^+ with which the isotope can exchange. However, Bradbury & Stulcova (1970) estimated the net losses to the blood by measuring the amount of ^{42}K that had remained in the brain during a period of ventriculo-cisternal perfusion; the amounts entering and leaving in the perfusion fluid were known, so by subtraction the amount leaving by the choroid plexuses and the blood vessels of the brain could be obtained. They found that the efflux of ^{42}K was, in fact, elevated by raising the concentration of inactive K^+ in the perfusion fluid. In Text-fig. 16 the outflux of K^+, determined by the estimated losses of ^{42}K, is plotted against the mean concentration of K^+ in the perfusion fluid. If a simple non-carrier mediated process were involved, the relation between outflux and concentration would be linear, but in fact it is S-shaped, with a steep rise between 3 and 6 m-equiv/l. indicating an acceleration of outflux with increasing concentration of K^+ over this range. The combination of an influx process, saturable at plasma concentrations above the normal level, with an efflux accelerated by c.s.f. concentrations above their normal level,

provides the basis for the remarkable homoeostasis of the composition of the c.s.f. with respect to K^+. It is likely that a similar combination of mechanisms operates across the blood–brain barrier, ensuring a homoeostasis of the level of K^+ in the extracellular fluid of the brain tissue (Bradbury, Segal & Wilson, 1972).



Text-fig. 16. Computed efflux of K^+ into the blood, during ventriculo-cisternal perfusion, as a function of the concentration of K^+ in the perfusion fluid (Bradbury & Stulcova, 1970).

To conclude, then, the blood–brain barrier is something very much more complex than a simple restraint on passage from blood to brain. Quantitatively it is reflected in variable rates of net flux of substances from blood into the tissue of the brain, rates that are governed by lipid solubility as with all cells of the body, and, superimposed on this, by carrier-mediated transport processes that are involved in maintaining the levels of many normal plasma constituents in the brain extracellular fluid at values that are independent, to a greater or less extent, of those in the plasma. In extreme cases, as with K^+, these mechanisms can achieve a virtually complete independence of plasma concentration, whilst in other cases, as with blood glucose, the extracellular fluid concentration, as indicated by the c.s.f. concentration, does in fact vary with the plasma concentration, so that in extreme hypoglycaemia, for example, the neurones suffer from glucose lack, and the so-called 'hypoglycaemic convulsions' are manifest. The removal of locally produced transmitters, once they have exerted their actions within the brain, does not, in general, rely on

active transport mechanisms, although these exist, since the local mechanisms of chemical change and uptake within synaptosomes seem adequate. Only with the prostaglandins, where chemical mechanisms of inactivation are absent, and local re-uptake mechanisms are likewise absent, is the active transport outwards an important, in fact the sole, mechanism for cessation of action.

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EXPLANATION OF PLATE

Light-micrograph of a transdural villus-like projection of the arachnoid mater; the subarachnoid space is filled with many blood corpuscles following a fresh subarachnoid haemorrhage. Glutaraldehyde and OsO_4. × 710 (Tripathi & Tripathi, 1974).

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(*Facing p.* 28)