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Professor R B Fisher (*Department of Biochemistry, University of Edinburgh*) was particularly interested in the pressure inside the cell, which was said to be at 25 atmospheres, this was about one molar, or about three times the ordinary isotonic pressure in a human being, and it was thus a high pressure, especially when dealing with the bombardment of a membrane. If one had these large hydrostatic pressure differences the kinetics were bound to be asymmetrical. He wondered whether anything had been done to reduce the osmotic pressure difference across the membrane and whether one would still have what had been called an 'active transport'.

Dr Wilson agreed that 25 atmospheres seemed to be a large pressure difference across the plasma membrane but the cells were so small that the actual pressure per square centimetre was not so very great. It was certainly greater than in animal cells which had, of course, the same osmotic pressure inside and out.

The question of whether transport could occur in cells with different internal osmotic pressures had not been studied at all systematically. In preliminary experiments Dr Wilson had found that in very hypotonic media the thiomethylgalactoside was efficiently transported and the rate was not greatly affected over a wide range of osmotic pressures.

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Transport in the Central Nervous System

Passage of material from blood to a tissue like skeletal muscle seems to be a fairly simple process governed largely by the speed with which material may escape from the capillaries into the extracellular space and thence into the cells; unless escape is very rapid indeed, the rate of blood flow is not a determining factor. If substances are chosen such that they pass very slowly, or not at all, into cells, or if their steady-state distribution between the intracellular and extracellular compartments favours a very low intracellular concentration, then the rate of accumulation in the tissue will be a simple function of capillary permeability, i.e. the ease of crossing from the lumen of the capillary to the surrounding extracellular space. The main experimental finding in this case is that substances escape at rates that are very nearly proportional to their diffusion coefficients in water. They behave as though they were passing through large water-filled pores. This means that there is no very great restraint on passage of such molecules and ions as sucrose, creatinine, Na^+ and Cl^-. Only when the molecule is very large, as with the plasma proteins and hæmoglobin, are the restraints considerable, so that it is customary to place the 'limiting pore size' of the capillary membrane at the diameter of the hæmoglobin molecule (about 70Å). Much larger molecules can, indeed, escape, but the quantities are so small that it is likely that they escape by a pinocytosis mechanism, or else through a few very large pores.

Passage of material from blood into brain is so different quantitatively from passage into other tissues that investigators are inclined to invoke a qualitative difference between the processes, a difference that is implied in the use of the term 'blood-brain barrier'; by this is meant that passage of the substances considered above, sucrose, creatinine, &c., out of blood into the substances of the brain and spinal cord is very much slower; furthermore, it is highly selective, so that whereas the passage of ^{24}Na into the tissue takes place at a measurable rate, the passage of sucrose and inulin is so slow that it is often difficult to measure appreciable uptake. Passage across the blood-brain barrier is thus slow and selective, and is reminiscent of transport across cell membranes. This analogy between blood-brain and cellular transport becomes more evident when substances of varying lipid solubility are compared. In the blood-brain system, increasing lipid solubility has a striking influence on the ease of crossing the barrier, so that the equilibration of brain with such anæsthetics as ether and chloroform, when these are injected into the blood, is virtually instantaneous. Lipid solubility favours passage out of capillaries into muscle, but here the effects are not so easy to assess because passage of lipid-insoluble substances is so rapid. It would seem, then, that materials passing from blood to brain must cross a protoplasmic layer, whereas passing from blood to muscle any protoplasmic barrier that exists can be easily circumvented, i.e. the barrier is permeated by large water-filled pores.

The blood-brain barrier, whatever its ultimate function, is important to the experimenter since, when he injects a drug into the blood, the failure to observe pharmacological effects may be due not so much to the absence of sensitive sites but to the failure of the drug to cross the blood-brain barrier sufficiently rapidly to enable the

build up of a pharmacologically active concentration at the site, e.g. the synaptic cleft. This probably accounts for the common failure to observe central effects when 5-hydroxytryptamine or curare is given intravenously.

The experimental study of the blood-brain barrier is not simple, owing to the complicating factor introduced by the presence of a circulating cerebrospinal fluid; this is secreted by the choroid plexuses in the ventricles and drained away into the dural venous sinuses; this latter process consists of an unrestricted flow through large holes in the arachnoid villi, so that all substances in the fluid may escape at the same rate by this route.

When a substance is injected into the blood, the rapidity with which a concentration can build up in the brain will depend not only on the blood-brain barrier but also on what happens in the cerebrospinal fluid; if it passes very rapidly into the CSF, then the concentration may build up here and allow an 'overspill' into the adjacent brain. If, on the other hand, passage is slow, it may happen that the CSF acts as a drain or sink, carrying away the substance from the brain and returning it to the blood in the bulk drainage process. Alternatively, the concentrations in the brain extracellular fluid and the CSF may rise at roughly the same rate, in which case there will be no adequate gradients favouring either overspill or sink-action. Experimentally we may find substances that fall into any one of the three categories; but the usual situation is that the CSF acts as a sink, preventing or retarding the build up of concentration in the brain. Thus it is very unlikely that the CSF acts as a source of nutrition for the brain; the blood-brain barrier restricts passage of glucose and amino acids from blood to brain, but it also does so from blood to CSF, so that the tissue must derive such quantities as are required mainly from the blood across the blood-brain barrier.

Thus we must seek some other function for the CSF than that of supplier of metabolites, and the observation that usually concentrations of certain solutes are maintained at a lower concentration in the CSF than in the extracellular fluid of the brain provides the clue. The CSF may, indeed, be a sink or drain, capable of removing unwanted solutes from the brain, whether these arise naturally through metabolism, or bacterial invasion, or whether they owe their presence to an initial passage across the blood-brain barrier. An excellent example of this process is shown by the thiocyanate ion; when it is injected into the blood the concentrations in CSF and brain remain very low for indefinite periods, and are always just a small fraction of the plasma concentration. The mechanism for this is twofold; first the choroid plexuses actively transport the ion out of the CSF into the blood, so that the concentration in this fluid remains very low. Second, the passage from blood to brain is restricted in rate, by operation of the blood-brain barrier; this allows the ion to pass into the tissue, but as fast as it enters it diffuses into the CSF and is carried back into the blood. The CSF is thus behaving analogously with the lymphatic system, providing a means of returning back to the blood, substances that have escaped from the blood. By raising the concentration of thiocyanate in the blood to a sufficiently high level, it is possible to 'saturate' the mechanisms whereby the choroid plexuses maintain the CSF concentration at a low value; as a result, the concentration in CSF rises and, in turn, that in the brain does so; it is when this concentration is reached that toxic manifestations occur. The same type of active process probably contributes to keeping low concentrations of noradrenaline in the brain.

The question now arises as to what is the anatomical basis for the restraint on passage from blood to brain that we describe as the blood-brain barrier? At present there is no unequivocal answer to this question, because we are still not certain of the anatomical basis for the unrestrained passage of material from blood to muscle. The latter system behaves as though the blood were separated from the adjacent extracellular space by a porous membrane with pores of 70Å diameter. The electronmicroscope has not revealed such pores, but only the much larger spaces between endothelial cells, probably 200Å wide or larger. These could not restrain the plasma proteins. If these channels are closed by tight-junctions, then the contents of the plasma must escape by crossing the endothelial cells, but if they did have to, one would not expect such indiscriminate and rapid permeability. Must we now invoke pinocytosis, the engulfment of plasma into vesicles which are subsequently emptied at the other side of the cell? This process would be too unselective, allowing molecules of different size such as plasma proteins, inulin and sucrose to cross at about the same rate, so we must invoke an additional porous structure on the outside to restrain the passage of the large molecules. This structure could possibly be the basement membrane. If we accept the hypothesis, then we must ask where the difference between a muscle and brain capillary lies. The striking feature of the brain capillary is its investment by protoplasmic processes of astrocytes, whilst it is rare to see vesicles in the cytoplasm of the endothelial cells. We may suppose, then, that in some way the presence of the astrocyte processes on the outer surface of the endothelial cells

inhibits the pinocytosis mechanism, so that material must pass through the endothelial cells. Having reached the highly porous basement membrane it may diffuse either between the astrocytes or through them, to pass eventually into the spaces surrounding the cells of the parenchyma, be they neurones or glia.

This is just one of a pair of hypotheses; it may well be that pinocytosis is an unimportant mechanism in the transport of solutes across the capillary, in which case we must seek some other mechanism, and thus some other basis for the difference between brain and muscle.

Finally, a few words about the function of those astrocytes that contribute to the covering of the capillary endothelium; it has been argued that they represent the pathway through which metabolites pass on their way to the neurones and other cells of the parenchyma; in other words that transport in the central nervous system is largely if not exclusively intracellular. This, in my view, is an unnecessary hypothesis; there is no doubt that in order to function as initiators and conductors of nerve impulses the neurones must be bathed by an extracellular fluid of high Na^+ and low K^+ concentrations, so that transport of solutes is much more likely to take place through this, although some intracellular movement will also occur. In my view these capillary astrocytes serve to maintain the composition of the extracellular fluid within a fixed range; thus, this fluid is undoubtedly in close diffusional relations with the CSF, so that any change in the composition of the one will be rapidly reflected in a change in the other. The CSF is not a simple filtrate from plasma; it is a specific secretion containing concentrations of K^+, Cl^-, Mg^{++} and Ca^{++} that are different from those to be expected of a simple filtrate, and therefore different from that expected of extracellular fluid as found in skeletal muscle. Thus we must expect the extracellular fluid of brain to be similar in composition to CSF, and it is difficult to see how this can be achieved other than by active processes on the part of cells that come into direct contact with the blood capillaries, i.e. the capillary astrocytes. Hence the blood-brain barrier is more than a restraint on rate of transport, it reflects the active processes that control exchanges of ions, as well as of metabolites, between blood and the extracellular fluid.

Dr I L Natoff (*Shell Research Laboratories, Sittingbourne, Kent*) asked whether Dr Davson had any views on the penetration of the brain by systemically administered compounds which might or might not bear formal positive charges. He was thinking particularly of atropine sulphate, the tertiary ammonium salt of the alkaloid, as opposed to atropine methonitrate, the quaternary ammonium salt. There was evidence that atropine sulphate could penetrate the brain and elicit central actions, whereas the methonitrate would not. Similarly, he noted that such amino acids as 5-hydroxytryptophan and 3:4,dihydroxyphenylalanine could exert centrally mediated effects when administered systemically, whereas the decarboxylated products had no central pharmacological activity.

Dr Davson, in reply, said that the fundamental thesis – that is the lipid solubility of the material – always held as far as the brain was concerned. If a substance was lipid soluble it was able to get out of the capillary into the adjacent extracellular spaces. The active transport process applied not only to substances like iodide, thiocyanate and penicillin, but also to some quaternary ammonium salts. It also included adrenaline, so that there was an active transport process for every base, especially the quaternary bases. Passage from the blood into the brain itself was very similar to the passage into any cell. It had already been seen that there was not only active transport but also this facilitative transfer which was highly specific. There was evidence that the same thing happened with the brain, that certain sugars could pass rapidly out of the capillary into the brain, but others could not. The same thing happened with amino acids, so the fact that one got quite specific differences among the amines was not surprising, whether by virtue of the action of the cerebrospinal fluid or of the brain itself.

Dr S P R Rose (*Imperial College, London*) referred to figures quoted by Dr Davson in his model for extracellular fluid and his calculation of the concentration in terms of extracellular fluid. He asked what figures Dr Davson was actually taking as the basis of the extracellular fluid.

Dr Davson replied that the actual volume of the extracellular spaces of the brain was now generally recognized and accepted by physiologists as 10–12% if the sucrose space had been measured under appropriate conditions.

Dr Rose therefore assumed that when the brain cerebral fluid space equilibrated with the cerebrospinal fluid, Dr Davson would take the volume to be 10%.

Dr Davson agreed.

Dr Rose asked whether Dr Davson was actually arguing that the extracellular fluid in the brain had been made by secretion from the astrocytes or from the capillaries.

Dr Davson said it could be either. He pointed out that the astrocytes had this characteristic of putting their little feet on top of the capillary, so that they were ideally situated for controlling the exit of material from the capillary. It could as well be argued that the capillary was secreting into a space between itself and the astrocyte and the material was going in between them.

Fundamentally the composition of the CSF was quite different from that of the blood. The potassium concentration was about 70% of that of plasma. When the CSF came into the ventricles and just off the surface of the brain, it had then been in the system for a long time and had been in contact with large areas of brain on its way. If the brain extracellular

fluid had had the same concentration of potassium as the blood, this fluid would have had a much higher concentration than the material just taken out of the ventricle. But in practice the potassium concentration actually fell, as the material stayed longer in contact with the brain. This applied not only to potassium but to other substances as well, so the only way to explain the fact that the composition of the CSF stayed pretty constant throughout the system (and it flowed fairly slowly and had a half-life of two or three hours) was that it was in contact with something very similar to itself. It was known that the CSF must be formed by active transport mechanisms so it was thought the extracellular fluid was also formed in this way.

Dr A M J N Blair (*Fisons Pharmaceuticals, Holmes Chapel, Cheshire*) said that pharmacologists were often interested in studying the effects of drugs on the central nervous system after either systemic or intracerebral administration. Occasionally a drug influenced behaviour only when very large doses were given systemically but intracerebrally very small doses were effective. He asked, if the difference in effects on the central nervous system between systemic and intracerebral administration was very great, whether it could be assumed that the drug did not cross the blood-brain barrier.

Dr Davson did not believe that the barrier was an absolute barrier in that sense of the term. Everything could get across, even proteins could get across from the blood to the brain. It was just a question of how high a concentration could be achieved in a given time. If the drug did not act in a fairly low concentration of blood, but acted in a high concentration, it simply meant that in the time it had been given it had not been possible with a low concentration to get enough absolute amount out.

Dr S J G Semple (*St Thomas's Hospital, London*) referred to the area postrema where the barrier might be different, and asked whether there was histological evidence that there might be pores in the capillaries. He also asked whether, if there were any anatomical difference, it would have any physiological significance.

Dr Davson said he had examined the electronmicroscopical literature but found it rather vague. People had shown quite large extracellular spaces in the area postrema and the other specialized parts where there was not much of a barrier, but he had not seen convincing evidence that the capillaries in those spaces were fundamentally different from those found elsewhere.

Dr Semple asked whether they had any physiological significance in the areas where the barrier was not so effective.

Dr Davson replied that he would like Dr Semple to answer the question himself as he was concerned with a possible physiological basis in the control of respiration. He asked Dr Semple whether, if he had a region where bicarbonate could easily escape from the blood into the brain, it would be a better place for the control of respiration.

Dr Semple replied that he did not know.

Professor A St G Huggett (*Edinburgh*) asked what was morphologically the exact location of the blood-brain barrier.

Dr Davson replied that morphologically they were in the hands of the anatomists; he could only repeat what they said, which was that a peculiar feature of the capillaries of the central nervous system was that they had these astrocyte covers, but the anatomists themselves did not agree that the covering was complete. If only 75% of the capillaries were covered and the other 25% uncovered, that could not act as a barrier. On the other hand, the basis of ordinary capillary permeability was not known.

Dr H O J Collier (*Parke Davis, Hounslow*) said that one feature of capillaries not in the brain was that their permeability could be increased by substances such as histamine and kinins. He asked whether this applied also to capillaries in the brain, and whether dyes would pass from the lumen of capillaries into the brain if kinins were administered along with the dyes into blood vessels.

Dr Davson said the blood-brain barrier was extraordinarily stable. He had never seen any demonstration that histamine caused any increase in permeability to materials such as trypan blue.

Dr E J M Campbell (*Royal Postgraduate Medical School, London*) found that recent publications had suggested that the H^+ concentration of CSF was relatively constant despite metabolic (non-respiratory) changes in the blood, and asked where this took place.

Dr Davson replied that the choroid plexus was continually secreting CSF with a bicarbonate composition of quite different concentration from that of the plasma. That was the main basis of the control, but what happened in the brain perimeter, where the same process was going on, was not known. It might be surmised that just because the concentration of bicarbonate in the CSF was characteristically different then the extracellular fluid in the brain might also have to have a similar composition.

Professor Eleanor Zaimis (*Royal Free Hospital, London*) said it had been suggested that the action of curare on the brain might be increased by histamine.

Dr Davson agreed that histamine caused the capillaries to dilate. In other parts of the body it opened the junctions and increased permeability. The action of histamine showed that the junctions between capillary membranes were reversible, but as he had said before there was no direct evidence to show that histamine increased capillary permeability in the brain.

Professor A S V Burgen
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The Effect of Drugs on Membrane Transport

I want to take up the story that Professor Ussing has told about active sodium transport in various tissues and examine the effects of pharmacological agents on it.

Let me first summarize what we want to know. One can regard the sodium pump as being con-