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# THE BLOOD-BRAIN BARRIER AND THE EXTRACELLULAR SPACE OF BRAIN

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When certain substances, e.g. thiocyanate, iodide, sucrose, etc., are injected into the blood stream, and their concentration in this fluid is maintained at a steady level, equilibration with the nervous tissue requires many hours or even days, whereas the equilibration of the same substances with the extracellular space of muscle is attained in a matter of seconds or minutes. It is this phenomenon that has given rise to the term blood–brain barrier, and has been attributed to the presence of exceptionally impermeable capillaries in nervous tissue, this impermeability being either a characteristic of the capillary endothelium or due to the presence of some other cellular layer, e.g. one composed of the processes of glial cells.

However, recent electron microscopical studies have indicated a virtual absence of an extracellular space in brain tissue (Wyckoff & Young, 1956; Schultz, Maynard & Pease, 1957; Horstmann, 1957; Farquhar & Hartmann, 1957). In consequence it has been argued, for example by Maynard, Schultz & Pease (1957) and by Edström (1958), that it is unnecessary to postulate the presence of any special barrier between blood and the tissue, since the very slow penetration of these typically 'extracellular' substances would be accounted for by the absence of an extracellular fluid for them to penetrate, such uptake as did occur representing penetration of cells, which would necessarily be very slow with these substances.

This argument can, however, be put to a very simple and unequivocal test. If the very slow uptake of thiocyanate, for example, were due solely to the circumstance that it had to penetrate cells, then clearly we should expect a similarly slow, or even slower uptake by excised tissue incubated in a Krebs-Ringer solution; by contrast, the uptake by excised muscle under the same conditions should be relatively rapid. In the present work, therefore, the penetration of $^{131}I$, sucrose and *p*-aminohippurate (PAH) into brain and muscle

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has been studied both *in vivo* and *in vitro*. The studies *in vivo* have provided quantitative information as to the extreme slowness with which these substances pass into the nervous tissue; whilst the studies *in vitro*, carried out on pieces of brain subjected to a minimum of damage (i.e. without slicing), have shown that penetration into this tissue is about as rapid as into muscle, and occurs to an extent that is commensurate with the presence of an extracellular space of at least 15% of the tissue. The results prove, therefore, that the concept of a blood–brain barrier is sound.

## METHODS

*Studies in vivo.* A definite plasma level of the substance to be examined was established by intravenous injection into the unanaesthetized rabbit, and maintained by continuous intravenous infusion with the apparatus described by Davson & Purvis (1952). At the appropriate time the rabbit was anaesthetized with pentobarbitone, a sample of cerebrospinal fluid was removed, and the animal was decapitated. The entire brain, less cerebellum, was removed, homogenized, and Somogyi filtrates of known amounts were made for the appropriate analysis or estimation of radioactivity. Since the amount of blood remaining in the brain might influence the apparent amount of substance penetrating, some experiments were carried out to estimate the average amount left. Blood cells were labelled with $^{51}Cr$ according to the method of Sterling & Gray (1950) and the amount remaining in the brain after decapitation was determined.

*Studies in vitro.* Pieces of otherwise intact cerebral hemisphere and of diaphragm from rabbits, removed under pentobarbitone anaesthesia, were used. The pieces of brain averaged 1 $cm^2$ in area; their thickness was on the average 2·3 mm whilst with diaphragm it was 1·5 mm. Incubation was carried out at 40° C under an atmosphere of 100% $O_2$ in a balanced Krebs–Ringer-phosphate medium (Krebs, 1950) which in addition contained, according to the experiment, $^{131}I$ in 2·5% NaI (not exceeding 7 parts of this solution to 93 parts of the Krebs medium); 4 parts of 12% sucrose to 16 parts Krebs medium; 100 mg PAH in 100 ml. of medium; $^{24}Na$ in isotonic NaCl-$NaHCO_3$ (not exceeding 2 parts of this solution to 98 parts of Krebs medium). When the outflux of chloride from the tissue was studied, the chlorides in the medium were replaced with nitrates. At varying intervals of time pieces were removed from the medium, rinsed for a few seconds in 12% sucrose (NaCl solution 0·9% (w/v) was used in the sucrose experiments); gently blotted, weighed and homogenized; Somogyi filtrates were made and the appropriate analysis or determination of radio-activity was carried out.

*Analytical.* Chloride was measured by the method of Sendroy (1937); sucrose by that of Hagedorn & Jensen (1923) before and after hydrolysis; PAH by the Bratton & Marshall (1939) technique. Radioactivity of $^{131}I$ and $^{24}Na$ was estimated in a liquid counter of the M6 (20th Century Electronics) type, corrections for resolving time, decay and background being made when necessary. The X-radiation of $^{51}Cr$ was determined with a well-type scintillation counter.

## RESULTS

### *Volume of blood in brain tissue*

On the average, the experiments with labelled erythrocytes indicated that 1·2 ml. of blood remained in each 100 g of brain; where necessary, therefore, a correction for this residual blood has been made for the experiments *in vivo*.

### *The barrier to PAH, $^{131}I$ and sucrose*

The very slow penetration of these substances from blood to brain is illustrated in Fig. 1 (left), where the 'space' occupied by the substance has

been plotted against time during which a constant concentration was maintained in the plasma. By 'space' is meant the volume of water required to dissolve the amount of substance found in 100 g of tissue to give a concentration equal to that in an ultrafiltrate of plasma. For example,

$$\text{chloride space} = \frac{\text{m-mole Cl/100 g tissue}}{\text{m-mole Cl/100 g plasma filtrate}} \times 100.$$

The figure shows that, with brain, this amounted to only about 1% of the tissue after 6 min, whilst after 2 hr it had only risen to 3–4%. The horizontal line indicates the chloride space, so that after 2 hr the system is very far from

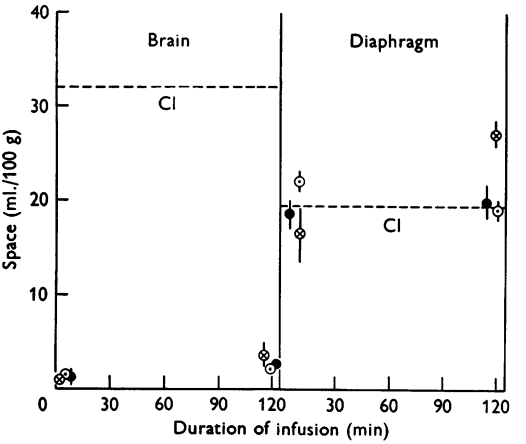


Fig. 1. Penetration of PAH, ⊗, sucrose, ●, and $^{131}I$, ⊙, into brain and diaphragm muscle of rabbits infused with the test substance for 6 or 120 min. The ordinate refers to the volume of tissue water (per 100 g tissue) that contains the same concentration of the test substance as in a plasma dialysate. Each point represents the average of determinations in 4–10 animals; S.E. shown as vertical lines.

TABLE 1. Values of the ratio (concn. in c.s.f.):(concn. in plasma) 120 min after establishing a constant concentration in the plasma; ±S.E.

| $^{24}Na$ | $^{131}I$ | Sucrose | PAH |
|---|---|---|---|
| 0·40±0·02 | 0·012±0·001 | 0·010±0·001 | 0·002±0·0005 |

achieving equilibrium with this space, if we suppose that this is possible. To the right of Fig. 1 are the results for penetration into muscle; once again the horizontal line indicates the chloride space. By contrast with brain there is a rapid equilibration, so that within about 6 min the volume of distribution corresponds approximately to the chloride space. Interestingly, after 2 hr there is some further penetration of PAH presumably into the cells of the muscle. In confirmation of earlier results (Davson, 1955), the rates of penetration of these substances into the cerebrospinal fluid were very slow, as is indicated by Table 1, where the penetration of $^{24}Na$ is included for comparison.

*Incubation experiments*

The uptake of $^{131}I$ and $^{24}Na$ by pieces of isolated brain is shown in Fig. 2, where the chloride space has also been plotted. The slow drift upwards of the chloride space indicates that the tissue was deteriorating a little, and it is presumably for this reason that the uptake of $^{24}Na$ goes beyond the normal limit observed in studies *in vivo*, namely to show a $^{24}Na$ space of 34·6% (Davson, 1955). The important point, however, is the rapid and smooth uptake of $^{131}I$, which contrasts so strongly with its negligible uptake *in vivo*. From the curve a fairly rough approximation to the effective diffusion coefficient of

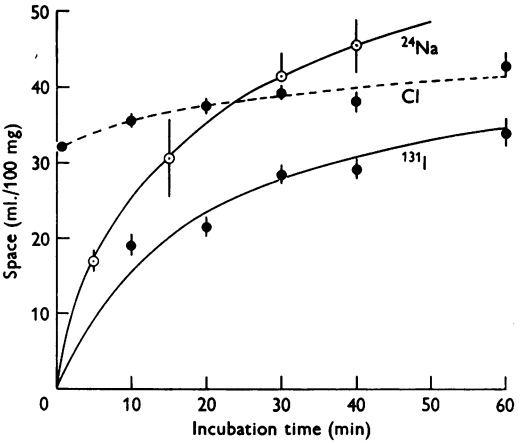


Fig. 2. Penetration of $^{24}Na$ and $^{131}I$, and changes in chloride space, in pieces of cerebral hemisphere incubated at 40° C. Ordinate as in Fig. 1. Each point represents the average of 6–10 incubations; S.E. shown as vertical lines.

$^{131}I$ in the tissue may be made. This comes out at $5 \times 10^{-6}$ cm²/sec, which compares with a value of $1{\cdot}2 \times 10^{-5}$ cm²/sec for free diffusion of NaCl in water. Under the same conditions, *in vitro*, $^{131}I$ penetrated diaphragm muscle in a comparable fashion, giving an apparent diffusion coefficient in this tissue of $3{\cdot}5 \times 10^{-6}$ (Fig. 3).

Brain or diaphragm, incubated in the presence of PAH, gave uptake curves of almost identical shape (Figs. 4 and 5), suggesting penetration into extracellular space associated with some penetration of cells. By contrast, the uptake of sucrose by the brain tissue (Fig. 4) ceased at an apparent equilibrium condition corresponding to a sucrose space of 15·5%. Sucrose continued to penetrate the diaphragm up to 40 min (Fig. 5), and we must conclude from this that some of this penetration, like that of PAH, was intracellular, possibly due to deterioration of the tissue.

The washing out of chloride from brain tissue was studied by soaking it in a medium in which cloride was replaced by nitrate. $^{131}I$ was present in the

medium so that the simultaneous uptake of this isotope could be measured. In Fig. 6, curve *A* represents the change of chloride space with time. It apparently approaches asymptotically a value of 10%. Curve *C* shows the

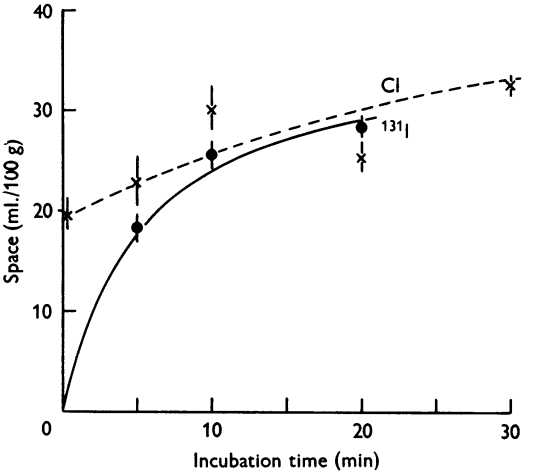


Fig. 3. Penetration of $^{131}I$, and changes in chloride space, in diaphragms incubated at 40° C; ordinate as in Fig. 1. Each point represents the average of 4–7 incubations. S.E. shown as vertical lines.

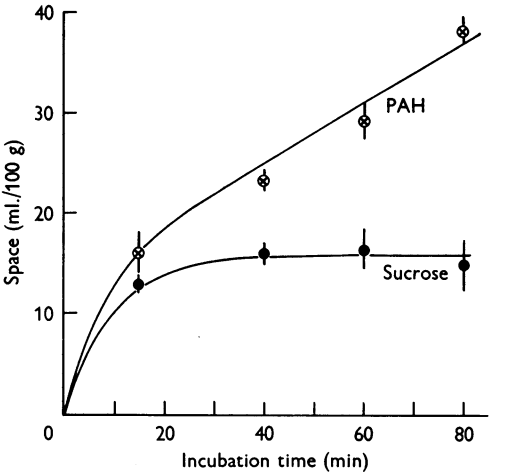


Fig. 4. Penetration of PAH and sucrose in pieces of cerebral hemisphere incubated at 40° C; ordinate as in Fig. 1. Each point represents the average of 4–9 incubations. S.E. shown as vertical lines.

uptake of $^{24}Na$ under the same conditions, whilst the dotted curve *B* shows how the chloride space *would* have decreased had all the chloride in the tissue been in the same compartment and had diffused out at a rate corresponding to the initial rate, i.e. with a computed coefficient of about $6 \times 10^{-6}$ cm$^2$/sec.

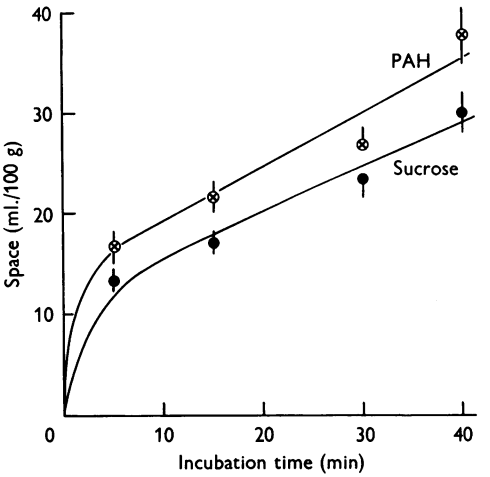


Fig. 5. Penetration of PAH and sucrose in diaphragm incubated at 40° C; ordinate as in Fig. 1. Each point represents the average of 4–10 incubations. S.E. shown as vertical lines.

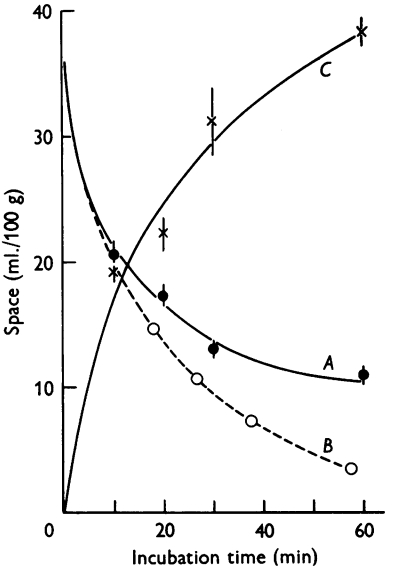


Fig. 6. Penetration of $^{131}I$ into (*C*) and the loss of chloride from (*A*), pieces of cerebral hemisphere incubated (at 40° C) in Krebs–Ringer-phosphate medium in which chloride was replaced by nitrate. Curve *B* represents the chloride loss expected if all the tissue chloride were equally diffusible. Ordinate as in Fig. 1. Each point represents the average of 8–12 incubations. S.E. shown as vertical lines.

## DISCUSSION

The present studies have demonstrated in the intact rabbit a marked blood-to-brain barrier to the passage of PAH, sucrose and $^{131}I$; thus supplementing several earlier investigations, e.g. those of Wallace & Brody (1939), who studied the penetration of iodide, bromide and thiocyanate into the brain of the dog. The uniform finding in the present work is that these substances achieve a volume of distribution in the brain that is only a small fraction of the chloride space. By contrast, in diaphragm muscle equilibrium at or near the level of the chloride space is attained within the first few minutes of infusion.

*In vitro*, however, the difference observed *in vivo* between brain and muscle disappears, $^{131}I$ diffusing into either at comparable rates that are not far different from those expected with free diffusion in water. Unlike the state of affairs during other studies of nervous tissue *in vitro* (e.g. McLennan, 1957), the brain was not cut into fine slices so that it cannot be argued that this uptake *in vitro* is due to penetration of damaged cells, or the opening up of pathways that would normally be closed *in vivo*. It is impossible to escape the conclusion, therefore, that there *is* a compartment in the brain into which such substances as $^{131}I$, sucrose and PAH could diffuse were there not some barrier between the blood and this compartment; that is to say there *is* an appreciable extracellular space, entry to which is impeded by a blood–brain barrier.

The only other point that may be profitably discussed here is the estimate of the volume of the extracellular space of brain that may be derived from the present studies. The chloride space found in most of the present studies, and in earlier work, is 32%, and this may be taken as an upper limit to the extracellular space. Washing-out experiments indicate, on the other hand, the presence of a residual amount of chloride corresponding to 10% of the tissue; if this represented intracellular chloride, it would mean that the true extracellular space was reduced by 10%, i.e. to 22%. This would imply, of course, that the chloride diffusing out rapidly was entirely extracellular but, in view of the manifest non-homogeneity of cerebral tissue, such a rigid separation of the chloride into two compartments is scarcely justified, and it may be that the electrolytes contained in the myelin, for example, may diffuse out more readily than those contained in axons (Shanes & Berman, 1955). Sucrose penetrates the nervous tissue to give a volume of distribution corresponding to a sucrose space of only 14–15%, and it seems fairest to take this as a lower limit, since this space is maintained during incubation for over an hour. It is interesting that under the same conditions the chloride and $^{24}Na$ spaces would have been abnormally high, about 42 and 50% respectively. Presumably the deterioration in condition of the tissue consists mainly in a passage

of sodium and chloride into the cells; if this is not accompanied by a corresponding loss of potassium, we may expect an increase in cellular water, with a possible contraction of the extracellular space, so that it may be that this is why the sucrose space appears so constant, the actual extracellular space decreasing with time, whilst equilibration with the outside fluid (inward diffusion of sucrose) proceeds. If this is true, the sucrose space does indeed correspond to a lower limit, and it might be concluded that the extracellular space—viewed as a compartment of the tissue into which diffusion from an outside medium takes place at a rate comparable to diffusion into the extracellular fluid of other tissues such as muscle—lies between 14 and 22%.

SUMMARY

1. The argument that the blood–brain barrier is due to the absence of an appreciable extracellular space, rather than to any impediment to escape from the capillaries, has been investigated.

2. It has been shown that substances such as iodide, *p*-aminohippurate and sucrose, when injected into the blood, penetrate brain tissue at barely measurable rates by contrast with their penetration into skeletal muscle. When penetration into isolated pieces of brain hemisphere from an incubation medium is studied, on the other hand, the uptakes are comparable in rate and amount with those in skeletal muscle. This proves that there is, indeed, a 'space' into which these substances can diffuse readily, so that failure of this space to be occupied in experiments *in vivo* indicates the presence of a barrier to diffusion from the vascular system.

3. Possible upper and lower limits to the extracellular space of brain are discussed in the light of the present studies.

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