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# A COMPARATIVE STUDY OF THE AQUEOUS HUMOUR AND CEREBROSPINAL FLUID IN THE RABBIT

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The aqueous humour and cerebrospinal fluid (c.s.f.) are specialized tissue fluids with a number of features in common. Just how far the analogy between the two fluids may be carried, however, is by no means clear, and in this work the two fluids, drawn from the same animal, have been compared with a view to establishing points of similarity and difference. The aspects investigated have been the distribution of certain solutes between the fluids and plasma, and kinetic studies of the blood-aqueous and blood-c.s.f. barriers. The interpretation of these kinetic studies required an investigation into the uptake of material by the lens from the aqueous humour, and a study of the kinetics of uptake of material by the brain tissue—the *blood-brain* barrier.

## METHODS

For the studies of the steady-state ratios, involving the use of isotopes in laboratory animals, these were injected intraperitoneally 24–48 hr before removal of the fluids. In general, after this period, the plasma concentration showed only small and inconsistent variations from hour to hour. Under pentobarbitone anaesthesia the blood, aqueous humour and c.s.f. were withdrawn, the last-named fluid by atlanto-occipital puncture, about 0·9 ml. being taken. Dialysates of plasma were made with the apparatus described earlier (Davson, Duke-Elder & Maurice, 1949). The plasma was dialysed against an equal volume of a mixture of isotonic NaCl and $NaHCO_3$ solutions (2:1) at pH 7·4; the substance of interest was added to the solution when necessary so as to give roughly the same concentration as that in the plasma when the steady-state distribution between plasma and aqueous humour or c.s.f. was determined; e.g. 8 mM-glucose, 1 mM-$^{82}$Br and 0·05 mM-$^{131}$I. Fluids from the horse were withdrawn under chloroform anaesthesia, with the kind assistance of Dr Paterson, Director of the Experimental Farm, C.D.E.E., Porton. The kinetic studies were carried out on adult Flemish Chinchilla rabbits with an average body weight of about 3 kg. No general anaesthesia was employed until the moment came for removal of the c.s.f. The general technique was similar to that described earlier (Davson & Matchett, 1953), a high plasma concentration of the substance studied being established by an initial intravenous injection of 10 ml. of an isotonic solution, and maintained by continuous infusion with a variable-speed injection machine (Davson & Purvis, 1952). During the course of the injection, blood samples were withdrawn, and at the appropriate moment the animal was anaesthetized, about 0·9 ml. of c.s.f.

withdrawn, and the anterior chamber of the eye emptied. When the lens and vitreous body were analysed the eye was enucleated, placed in solid $CO_2$, and dissected when frozen. The isotopes employed were received from Harwell in the following forms: $^{24}Na_2CO_3$; $^{42}KHCO_3$; $NH_4{}^{82}Br$ (this contained the short-lived isotope $^{80}Br$ as well); and carrier-free $^{131}I$ which, for the purposes of injection, was made up in a mixture of isotonic solutions of NaI and NaCl in the proportion of 30 to 70, so as to minimize losses to the thyroid. When the analytical method required the use of a protein-free filtrate, all three fluids were submitted to the same procedure. When brain tissue was analysed, a Somogyi-filtrate was prepared from about 2 g of the whole minced brain. Isotopes were estimated in either liquid counters of the M 6 (20th Century Electronics) type or by the window-type (G.E.C.-C.V. 2138) in nickel trays containing 1 ml. of water plus 0·1 ml. of the fluid to be examined. Corrections for resolving time, decay and background were made when necessary. Chloride determinations were usually made by the Volhard method, the titration being carried out after filtering off the AgCl precipitate through a Gooch crucible; for studies on the brain filtrate, however, the Sendroy (1937) technique was employed. Sodium was determined by the Barber & Kolthoff (1928) method as described earlier (Davson, 1939); thiocyanate by the method of Aldridge (1945); creatinine by the Folin-Wu method (1919); thiourea and its derivatives by the modified method of Chesley (1944) described earlier (Davson & Matchett, 1953), the phosphate buffer, however, being replaced by a citrate-HCl mixture to permit of the use of Somogyi filtrates of brain and lens. Glucose was determined by the Hagedorn & Jensen (1923) method and sucrose by the same method, after hydrolysis; *p*-amino-hippurate was determined by the method of Bratton & Marshall (1939); and ascorbic acid by titration of 1 ml. samples in acetic-metaphosphoric acid with 2:4-dichlorophenol-indophenol. In the calculation of the results, corrections were applied for the greater specific gravity of plasma; for dilution errors due to the presence of heparin solution in the blood-syringes; and, in the kinetic studies, for the high plasma concentration during the first few minutes of injection, and for any adsorption of the penetrating solute by the plasma proteins. Conductivity of the aqueous humour and c.s.f. was determined with an impedance-comparison meter at a frequency of 10,000 c/s, the fluids being drawn into a conductivity cell, which was made out of a 1 ml. pipette by inserting two electrodes into the bulb; the pipette was completely submerged in melting ice in a Dewar flask. Depression of freezing-point was measured with the apparatus recently described (Davson & Purvis, 1954).

The chemical and counting errors were rarely greater than 1%, except where the concentration in the fluid was very low (e.g. *p*-amino-hippurate in the c.s.f.). In general, analytical errors were overshadowed by the animal-to-animal variation. The total number of animals used for the kinetic study of any substance is mentioned in the legends to the figures describing the results. The coefficient of variation for the values of the concentration-ratios shown in the graphs varied between 2 and 10%.

## RESULTS

### *Distributions, conductivity and total osmotic concentrations*

In Table 1 are shown the distributions of $Cl^-$, $^{24}Na$, $^{82}Br$, $^{42}K$, $^{131}I$, glucose and ascorbic acid, between plasma and aqueous humour ($r_{Aq}$) and plasma and c.s.f. ($r_{Csf}$), where $r$ is the ratio: Concentration in fluid water/Concentration in plasma water. In the same table are included values for the distributions between plasma and its dialysate ($r_{Dial}$), so that deviation from thermodynamic equilibrium can be readily ascertained, an excess of a substance in the aqueous humour or c.s.f. being indicated by a value of $r$ greater than $r_{Dial}$. The values of $r_{Aq}$ and particularly of $r_{Csf}$, for $^{131}I$, were very variable and the limits rather than the means are presented. It will be seen that there is a slight deficiency of chloride in the aqueous humour by comparison with a dialysate, and a large

deficiency of iodide, but a small excess of sodium. In the c.s.f. the excess of sodium is considerably larger, the concentration being some 9% greater than that required by a Donnan distribution; chloride, too, is in large excess (16%), whereas there are large deficiencies of potassium, bromide and iodide, in agreement with earlier findings (see, for example, Kral, Stary & Winternitz, 1929; Wallace & Brodie 1940). The concentration of the reduced form of ascorbic acid is very much higher in the aqueous humour than in the c.s.f. No attempt has been made to compute distribution ratios with this substance as the concentration of the reduced form in the plasma is said to be negligibly small (Plaut & Bülow, 1935). The total reducing values of both aqueous humour and c.s.f. are considerably less than in the plasma; the much greater deficiency in the c.s.f. is nearly equal to that observed in the vitreous body (Davson & Duke-Elder, 1948). The large difference in sodium and chloride concentrations

TABLE 1. Distributions of various substances between aqueous humour and plasma ($r_{Aq}$), c.s.f. and plasma ($r_{Csf}$), and plasma-dialysate and plasma ($r_{Dial}$), where $r$ represents the ratio: concentration in fluid water/concentration in plasma water

| Substance | $r_{Aq}$ | $r_{Csf}$ | $r_{Dial}$ |
|---|---|---|---|
| $^{24}Na$ | 0·96±0·01 | 1·03±0·005 | 0·945±0·003 |
| $^{42}K$ | 0·955±0·02 (5) | 0·52±0·04 (5) | 0·96±0·005 |
| Cl | 1·015±0·01 | 1·21±0·007 | 1·04±0·006 |
| $^{82}Br$ | 0·98±0·015 | 0·715±0·02 | 0·96±0·01 (3) |
| $^{131}I$ | 0·32–0·51 (5) | 0·004–0·04 (4) | 0·85±0·01 (4) |
| Glucose | 0·86±0·025 | 0·64±0·02 | 0·97±0·01 |
| Ascorbic acid | 18·5 mg/100 ml. | 1·55 mg/100 ml. | — |

With ascorbic acid the concentrations in aqueous humour and c.s.f. have been presented, as it is doubtful whether the reduced form of this substance is present in the plasma. Limits are standard errors. Numbers in brackets are numbers of experiments where these were less than six.

between c.s.f. and aqueous humour might be expected to be reflected in a difference in electrical conductivity and in total osmolar concentration, as determined by depression of freezing-point; in studies on six rabbits the average difference in conductivity was 3·8% (±0·3 S.E.), the c.s.f. having the larger value, and the difference of total osmolar concentration was 3·5% (±0·11 S.E.), the c.s.f. being the more concentrated solution. The large difference in chloride concentration between c.s.f. and aqueous humour in the rabbit follows from the circumstance that the distribution of this ion between plasma and aqueous humour is close to that required by the Donnan equilibrium, whereas the distribution between plasma and c.s.f. differs markedly from this requirement. In other species, however, the distribution of chloride between plasma and aqueous humour may diverge very widely from the Donnan distribution (Davson, Matchett & Roberts, 1952), $r_{Aq}$ having a value of 0·935 in the guinea-pig, lying at one extreme of the series, and a value of 1·14 in the horse, at the other end. In Table 2 the values of $r_{Aq}$, $r_{Csf}$ and $r_{Dial}$ for both sodium and chloride in a number of species are presented. It will be seen that the chloride and sodium distributions between plasma and c.s.f. do not vary greatly from

species to species; the same is true for the sodium distribution between plasma and aqueous humour, so that it is essentially the chloride distribution between plasma and aqueous humour that shows a very large species-to-species variation.

*Effect of age on chloride distribution.* During studies on the chloride distribution between the aqueous humour and plasma of different species, a variability was encountered which was traced to the use of immature animals. In all the species studied, the chloride distribution was closer to a Donnan distribution in the immature animal. Thus young puppies of 3–5 months had an average value of $r_{Aq}$ equal to 1·03; 3 calves of 10–28 days a ratio of 1·09; these may be compared with values of 1·07 and 1·13 in the respective adult animals.

TABLE 2. Distributions of chloride and sodium between aqueous humour and plasma ($r_{Aq}$), c.s.f. and plasma ($r_{Csf}$), and plasma-dialysate and plasma ($r_{Dial}$), in various species

| | Chloride | | | Sodium | | |
|---|---|---|---|---|---|---|
| Species | $r_{Aq}$ | $r_{Csf}$ | $r_{Dial}$ | $r_{Aq}$ | $r_{Csf}$ | $r_{Dial}$ |
| Horse | 1·14 (5) | 1·19 (3) | 1·05 (3) | 0·935 (3) | 0·97 (1) | 0·925 (1) |
| Dog | 1·07 | 1·11 | 1·02 | 0·96 (5) | 0·975 (5) | 0·945 |
| Cat | 1·055 (4) | 1·15 (4) | 1·03 | 0·98* (3) | 1·01* (3) | 0·935 |
| Rabbit | 1·01 | 1·21 | 1·04 | 0·96* | 1·03* | 0·945* |
| Guinea-pig | 0·935 | 1·18 | 1·02 | 0·98* | 1·04* | 0·955* |

Asterisks indicate that the distributions were determined with $^{24}Na$. Numbers in brackets indicate number of experiments where these were less than six. In all cases, c.s.f. and aqueous humour were from the same animals. Values for $r_{Dial}$ for cat and dog taken from earlier work (Davson, Duke-Elder & Maurice, 1949).

## *Penetration of non-electrolytes and ions into aqueous humour and cerebrospinal fluid*

*Note on terminology.* By the expression 'rate of penetration' we imply a permeability constant, i.e. the number of molecules passing unit area of a separating membrane in unit time with unit difference of concentration. In the present kinetic studies the variables measured are a ratio of concentrations, e.g. $C_{Aq}/C_{Pl}$, and time; without a knowledge of the area-to-volume relationship of the system, it is impossible to deduce a true permeability constant for any penetrating substance. If a single system is studied, however, a transfer constant of dimension $t^{-1}$ may frequently be deduced from the curve of $C_{Fluid}/C_{Pl}$ against time, and this will be proportional to the true rate of penetration or permeability constant. Thus, inspection of a series of curves of $C_{Fluid}/C_{Pl}$ against time will enable us to state qualitatively that the rates of penetration of the substances concerned are in a certain order, and the computation of transfer constants, where possible, will provide a measure of the differences in permeability. When two different systems are compared, however, the differing area-to-volume relationships will preclude any deductions as to the rates of penetration of given substances into the separate fluids, and a comparison of transfer constants in the two systems permits only the statement that the rates of approach to equilibrium, or the steady-state, are greater or less in the one system than in the other. In the following description, therefore, the expression 'rate of equilibration' has been used wherever comparisons between the two systems have been made, and the use of the term 'rate of penetration' has been confined to those instances where it is clear from the context that we are concerned with relative rates of penetration into the same fluid.

In the present work the simultaneous penetration of the following substances into both aqueous humour and cerebrospinal fluid has been studied: $^{24}Na$,

$^{82}Br$, $^{131}I$, $^{42}K$, $CNS^-$, thiourea, methyl thiourea, ethyl thiourea, propyl thiourea, ethyl alcohol, creatinine, glucose, sucrose and *p*-amino-hippurate. $^{24}Na$ and thiourea were studied together in the same animal, so that any comparisons between these two substances are exceptionally accurate. The results for most of the substances studied are presented in Figs. 1–3, where the ratio:

$$\frac{\text{Concentration in fluid } (C_{Aq} \text{ or } C_{Csf})}{\text{Concentration in plasma } (C_{Pl})} \times 100$$

has been plotted against time. (For propyl thiourea only one point is presented as this substance is no longer manufactured, and the amount available was only adequate for nine experiments.)

A comparison of the sets of curves in the figures indicates, in general, that the relative rates of equilibration of plasma with aqueous humour, on the one hand, and of plasma with c.s.f. on the other, bear some relation to the absolute rates. Thus the equilibration of creatinine between plasma and aqueous humour is relatively slow, but more rapid than the corresponding process in the c.s.f. Thiourea equilibrates more rapidly, and the discrepancy between the rates is smaller; methyl thiourea equilibrates still more rapidly, and now it is difficult to say, on inspection, whether there is any discrepancy at all; finally, with ethyl alcohol, the most rapidly equilibrating substance studied, the discrepancy has changed sign, equilibration between plasma and c.s.f. being obviously the more rapid process.

With substances that penetrate very slowly into the c.s.f., such as sucrose, *p*-amino-hippurate, thiocyanate, and iodide, it was not considered profitable to study penetration over many periods of time, owing to the larger animal-to-animal variability, and the greater experimental errors involved in the determination of the small amounts of material that penetrate the c.s.f. Instead, the values of $C_{Aq}/C_{Pl}$ and $C_{Csf}/C_{Pl}$ after 120 min have been tabulated (Table 3). It will be seen that with these very slowly penetrating substances the general trend is obvious, the smaller the concentration found in the fluid after 120 min the bigger the discrepancy between aqueous humour and c.s.f. No further quantitative significance should be attached to the results on $^{131}I$ and thiocyanate, however, since the variability encountered was unusually high and was probably due to the intervention of some metabolic process in the brain tissue.

*Penetration into the lens.* It will be noted from Fig. 1 that, with the slowly penetrating substance, creatinine, the value approached by $C_{Aq}/C_{Pl}$ at larger values of $t$ is markedly different from unity; as the speed of penetration of a substance increases, this limiting value approaches unity. With the c.s.f., the same phenomenon is observed (Fig. 2), but the steady-state ratio of unity is certainly achieved with methyl thiourea, and is possibly approached by the more slowly penetrating thiourea, although in the aqueous humour the corresponding values, estimated by a logarithmic extrapolation, are about 0·925

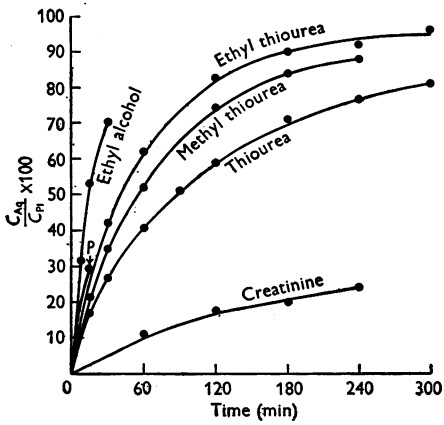


Fig. 1

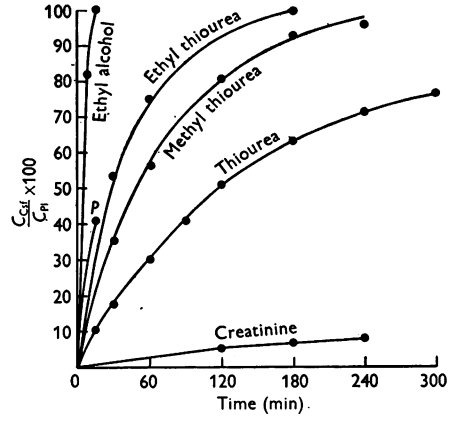


Fig. 2

Fig. 1. Penetration of some non-electrolytes into the aqueous humour.

Ordinates: $\frac{\text{concentration in aqueous humour } (C_{Aq})}{\text{concentration in plasma } (C_{Pl})} \times 100.$

Abscissae: time in min.

(Numbers of animals used: ethyl alcohol, 21; propyl thiourea, 9; ethyl thiourea, 41; methyl thiourea, 32; thiourea, 82; creatinine, 23. *P* represents propyl thiourea.)

Fig. 2. Penetration of some non-electrolytes into the c.s.f.

Ordinates: $\frac{\text{concentration in c.s.f. } (C_{Csf})}{\text{concentration in plasma } (C_{Pl})} \times 100.$

Abscissae: time in min.

(Numbers of animals used same as for Fig. 1. *P* represents propyl thiourea.)

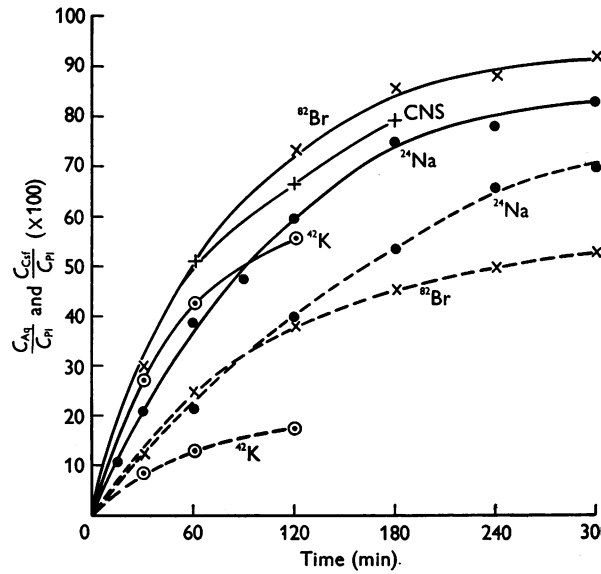


Fig. 3. Penetration of some ions into aqueous humour (full lines) and c.s.f. (broken lines). Ordinates and abscissae as before. (Numbers of animals used: $^{82}$Br, 35; CNS, 20; $^{42}$K, 18; $^{24}$Na, 75.)

and 0·85 respectively. It was considered that these relatively small discrepancies of the limiting values of $C_{Aq}/C_{Pl}$ from unity, observed with the thioureas (as opposed to the larger ones observed with creatinine, etc.) were due to penetration into the lens which, in the rabbit, has about twice the

TABLE 3. The equilibration of slowly penetrating substances. $C_{Aq}$ and $C_{Csf}$ are the concentrations in the aqueous humour and c.s.f. respectively, 120 min after a constant concentration, $C_{Pl}$, had been established in the plasma

| Substance | $C_{Aq}/C_{Pl}$ | $C_{Csf}/C_{Pl}$ |
|---|---|---|
| Thiocyanate | 0·67±0·035 | 0·095±0·06 |
| Iodide | 0·48±0·03 | 0·018±0·03 |
| Creatinine | 0·176±0·008 | 0·055±0·006 |
| Sucrose | 0·070±0·008 | 0·022±0·004 |
| *p*-Amino-hippurate | 0·070±0·005 | 0·0035±0·0005 |

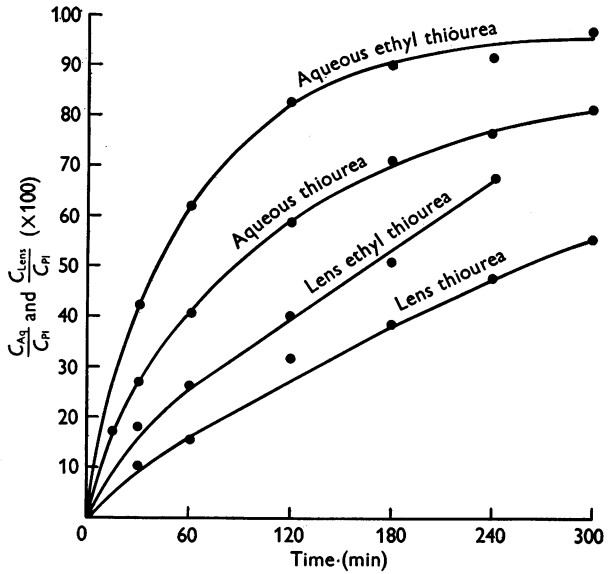


Fig. 4. Penetration of thiourea and ethyl thiourea into aqueous humour and lens.

Ordinates: $\frac{\text{concentration in aqueous humour } (C_{Aq}) \text{ or lens } (C_{Lens})}{\text{concentration in plasma } (C_{Pl})} \times 100.$

Abscissae: time in min.

(Curves for penetration into aqueous humour are the same as in Fig. 1. Points for the lens curves represent means of not less than six lenses.)

weight of the aqueous humour. That this could be a significant factor was shown by analyses of lenses taken from eyes at different stages in equilibration; thus Fig. 4 shows the penetration of thiourea and ethyl thiourea into the aqueous humour and lens; the fact that even after 5 hr the concentration in the lens is considerably less than that in the aqueous humour shows that the loss of material to the lens must be a powerful factor in determining the slope

of the curve of $C_{Aq}/C_{Pl}$ against time at the later stages of equilibration. According to the view developed earlier (Davson & Matchett, 1953), the great bulk of a substance such as thiourea would penetrate the anterior chamber by flow through the pupil, a relatively small proportion entering by diffusion across the iris. If this were true, we should expect the uptake by the lens to take place mainly during the flow through the pupil, rather than by a back-diffusion from the anterior chamber. In the former case the uptake should be independent of pupillary area, whilst in the latter it should vary linearly with it. By treating one eye of an animal with atropine and the other with pilocarpine, a difference in pupillary area of about 16-fold was obtained, yet the amounts of thiourea penetrating the two lenses were approximately equal, the eye with the smaller pupil actually having the greater amount owing to the action of pilocarpine, which slightly increases the rate of formation of the aqueous humour. That the thiourea taken up by the lens comes predominantly from the aqueous humour, as opposed to diffusion from the vitreous body, was shown by sectioning the frozen lens equatorially; in all cases studied, the anterior half contained about twice as much as the posterior half.

*Penetration into the vitreous body.* An analysis of the curve of penetration of $^{24}Na$ into the aqueous humour indicates that it approaches a steady-state ratio of about 0·89; in this case there is no doubt that the steady-state level is temporary, since after 24–48 hr the ratio is 0·96 (Table 1); diffusion of this isotope into the lens during the course of equilibration of plasma with aqueous humour is unlikely to be a significant factor, since the concentration of sodium in the lens is very small (Langham & Davson, 1949); loss to the vitreous body, however, is likely to be very significant since diffusion of sodium into this body from the choroid is negligible (Davson, Duke-Elder, Maurice, Ross & Woodin, 1949), at any rate in the cat; it therefore seems likely, as Maurice (1951) has argued, that the temporary steady-state approached by $^{24}Na$ is due predominantly to loss to the vitreous body. In the course of this work a number of analyses of lens and vitreous body have been carried out, at different stages of equilibration with the plasma, during kinetic experiments with $^{24}Na$, $^{42}K$, thiourea, and ethyl thiourea. The results are presented in Table 4, in which the values of the ratios: $C_{Aq}/C_{Pl}$, $C_{Vitreous}/C_{Pl}$ and $C_{Lens}/C_{Pl}$, attained after the stated intervals, are given. With $^{24}Na$ it is clear that losses to the lens are not significant; with $^{42}K$, thiourea and ethyl thiourea they are very significant. Equilibration of the vitreous body almost keeps pace with that of the aqueous humour in the case of ethyl thiourea, but lags behind in the case of the other substances, as a result, presumably, of the diminishing contribution of diffusion from the choroid and retinal capillaries. With $^{24}Na$ and $^{42}K$, therefore, losses to the vitreous body will be relatively large; moreover, since $^{42}K$ is also taken up strongly by the lens, we may expect a much more serious deviation from a simple logarithmic course of penetration than with $^{24}Na$.

TABLE 4. Equilibration of aqueous humour, lens and vitreous body with the blood plasma. $C_{Aq}$, $C_{Vitr}$ and $C_{Lens}$ are the concentrations in the aqueous humour, vitreous body and lens, respectively, at the stated interval after a constant concentration, $C_{Pl}$, had been established in the blood plasma. Concentrations computed as weights per unit weight of water.

| Substance | $t$ (min) | $\frac{C_{Aq}}{C_{Pl}}$ | $\frac{C_{Vitr}}{C_{Pl}}$ | $\frac{C_{Lens}}{C_{Pl}}$ |
|---|---|---|---|---|
| Thiocyanate | 120 | 0·665 | 0·28 | 0·185 |
| Iodide | 120 | 0·48 | 0·125 | — |
| Thiourea | 120 | 0·59 | 0·432 | 0·32 |
| | 180 | 0·71 | 0·59 | 0·385 |
| | 240 | 0·77 | 0·73 | 0·475 |
| | 300 | 0·81 | 0·74 | 0·56 |
| Ethyl thiourea | 30 | 0·42 | 0·335 | 0·18 |
| | 60 | 0·62 | 0·57 | 0·26 |
| | 180 | 0·90 | 0·89 | 0·51 |
| $^{42}K$ | 60 | 0·425 | 0·10 | 0·43 |
| | 120 | 0·56 | 0·19 | 0·99 |
| $^{24}Na$ | 120 | 0·60 | 0·165 | — |
| | 180 | 0·75 | 0·23 | 0·026 |
| | 240 | 0·78 | 0·35 | — |
| | 300 | 0·83 | 0·405 | 0·05 |
| $^{82}Br$ | 120 | 0·745 | 0·16 | — |

*Penetration of glucose*

The measurement of the penetration of glucose into the c.s.f. is not easy, since a knowledge of the concentration originally present is required; this would involve a preliminary withdrawal of fluid which would most probably upset the normal pressure relationships. An approximate measure can be obtained, however, from the concentration in the aqueous humour; thus the average of ten experiments in which both fluids were withdrawn simultaneously gave a value of 0·74 for the ratio: $C_{Csf}/C_{Aq}$. The experimental procedure consisted, therefore, of withdrawing one aqueous humour; establishing a high concentration of glucose in the plasma, and withdrawing the second aqueous humour and the c.s.f. after 60 min. The results of seven experiments gave the following mean figures ($C_{Pl}$ being given the arbitrary value of 100):

| $C_{Pl}$ | $C_{Aq^1}$ | $C_{Aq^2}$ | $C_{Csf^1}$ | $C_{Csf^2}$ |
|---|---|---|---|---|
| 100 | 35 | 66 | 26 | 41·5 |

*Kinetics of penetration into the brain tissue*

The c.s.f. is in close diffusional relationship with the cerebral tissue, which is supplied by the cerebral capillaries; a substance in the blood may presumably pass into the c.s.f. by way of the choroid plexuses; further possible routes are a direct diffusion from the vessels of the pia, and finally, a less direct diffusion from the cerebral capillaries by way of the cerebral extracellular fluid; alternatively, the concentration gradients may be favourable for diffusion from the c.s.f. into the cerebral extracellular fluid. To obtain evidence of the concentration relationships during equilibration with a constant plasma concentration,

the brain was excised, after removal of the c.s.f., and both were analysed. For the interpretation of the results an approximate estimate of the extracellular volume is required, and for this purpose the 'chloride-space' and 'sodium-space' were determined. Animals were injected with $^{24}Na$ and 48 hr later a blood sample was taken, the c.s.f. was withdrawn and the brains treated as above. The chloride- and $^{24}Na$-spaces were calculated as the weights of fluid that would contain the observed amounts of chloride or $^{24}Na$ in 100 g of tissue at a concentration corresponding to that in a dialysate of plasma; the values obtained were 31·4% ± 0·8 and 34·6% ± 0·8 respectively. Since these figures

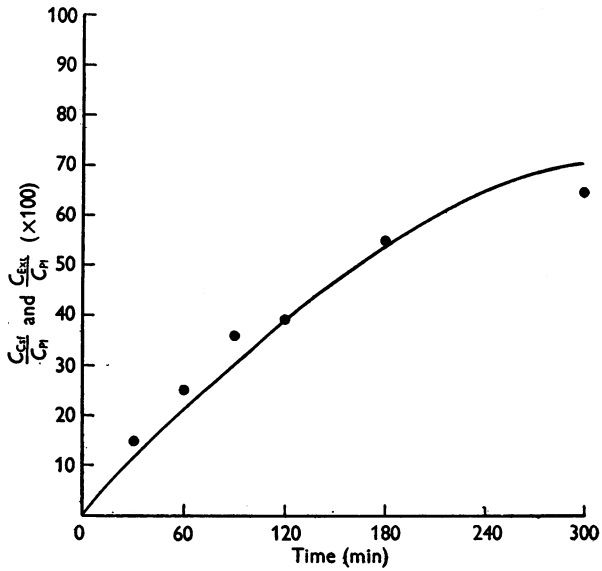


Fig. 5. Penetration of $^{24}Na$ into c.s.f. (smooth curve) and brain extracellular $H_2O$ (points).

Ordinates: $\frac{\text{concentration in c.s.f. } (C_{Csf}) \text{ or brain extracellular } H_2O \ (C_{Ext})}{\text{concentration in plasma } (C_{Pl})} \times 100.$

Abscissae: time in min.

(Curve for $^{24}Na$ is the same as in Fig. 3. Points are mean figures derived from a total of twenty-one brain analyses.)

must represent upper limits to the actual extracellular space, the chloride-space, being the smaller, has been chosen. In calculating the results of kinetic experiments on the penetration of $^{24}Na$ it may be assumed, as a first approximation, that penetration is confined to this compartment of the brain; on this basis, the points plotted in Fig. 5 have been computed, the smooth curve being the average course of penetration of $^{24}Na$ into the c.s.f. It will be seen that the points lie sufficiently close to the curve to suggest that the blood-c.s.f. and the blood-brain barriers are very similar in respect to sodium, in the sense that the rate of equilibration of the c.s.f. with plasma is approximately equal to the rate

of equilibration of the brain extracellular fluid with plasma. A similar conclusion was reached by Wallace & Brodie (1939) with respect to the anions $Br^-$, $I^-$ and $CNS^-$, and in the course of this work some studies with $^{82}Br$ were in excellent agreement. With the remaining substances studied, namely creatinine, thiourea, methyl thiourea, ethyl thiourea, propyl thiourea and ethyl alcohol, the presentation of the results is not so simple because the substances will penetrate the cells of the tissue at increasing rates. Since we are concerned chiefly with the concentration relationships between c.s.f. and extracellular fluid, it is sufficient to compute upper and lower limits to the

TABLE 5. Equilibration of c.s.f. and brain tissue with the plasma. $C_{Csf}$ is the concentration in the c.s.f. at the stated time after a constant concentration of the substance, $C_{Pl}$, had been established in the plasma. $C_{Ext}$ is the concentration that would be found if the substance were confined to the chloride-space, and $C_{H_2O}$ the concentration if it were equally distributed throughout the brain water.

| Substance | $t$ (min) | $\frac{C_{Csf}}{C_{Pl}}$ | $\frac{C_{Ext}}{C_{Pl}}$ | $\frac{C_{H_2O}}{C_{Pl}}$ |
|---|---|---|---|---|
| Creatinine | 180 | 0·08 | 0·16 | 0·07 |
| Thiourea | 90 | 0·44 | >1 | 0·52 |
| | 180 | 0·65 | >1 | 0·78 |
| | 300 | 0·74 | >1 | 0·84 |
| Methyl thiourea | 60 | 0·56 | >1 | 0·82 |
| | 120 | 0·79 | >1 | 1·04 |
| Ethyl thiourea | 30 | 0·54 | >1 | 0·76 |
| | 60 | 0·86 | >1 | 1·04 |
| Propyl thiourea | 15 | 0·42 | >1 | 0·81 |
| Ethyl alcohol | 3 | — | >1 | 0·96 |
| | 7 | 0·78 | >1 | 0·93 |

concentration of penetrating material in the extracellular fluid; these limits will be given by assuming that (*a*) all the material in the brain is in the extracellular fluid and (*b*) that all the material in the brain is equally distributed throughout the brain water (77·1 % by weight). The actual concentration will lie between these values. In Table 5, the results of computations carried out in this way are presented. We may conclude first, that as lipoid solubility increases from creatinine to ethyl alcohol, the rate of penetration of the blood-brain barrier increases, just as with the blood-aqueous and blood-c.s.f. barriers. Furthermore, it is clear from Table 5 that equilibration with the extracellular fluid is more rapid than equilibration with the c.s.f. Thus, after maintaining a constant concentration of thiourea in the blood for 90 min, the concentration in the c.s.f. was 44 % of this; the *minimum* concentration in the extracellular fluid was 52 %. With ethyl thiourea, after 30 min, the concentration in the c.s.f. was 54 % of the constant plasma concentration, whilst the *minimum* extracellular concentration was 76 %. The equilibration of alcohol with the brain tissue was so rapid that its rate was not measurable; thus, after 3 min, the minimum concentration was 96 % comparing with a c.s.f. concentration of about 35 % (Fig. 2); this value of 96 % may very well be low, however

owing to possible losses of ethyl alcohol during manipulation of the brain tissue (to minimize these losses, the brain was immediately frozen in solid $CO_2$). This very rapid penetration of ethyl alcohol into the brain tissue is consistent with the finding of Bering (1952) that the exchange of $D_2O$ between plasma and brain is complete in a few sec. We may conclude from these results on non-electrolytes that, for the substances considered, when a constant concentration is maintained in the plasma, the concentrations in c.s.f. and brain extracellular fluid will be such that diffusion will be from the latter to the c.s.f.

*Variation with age*

The rate of penetration of the water-soluble *p*-amino-hippurate ion into the aqueous humour is probably a measure of the 'leakiness' of the barrier separating it from blood, its penetration being probably by filtration through intercellular spaces (Davson, 1953); it is therefore of interest to see how this rate changes with age of the animal. A litter of rabbits, 6 weeks old when

TABLE 6. Changes in the blood–aqueous and blood–c.s.f. barriers with age. $C_{Aq}$ and $C_{Csf}$ are the concentrations of *p*-amino-hippurate in the aqueous humour and c.s.f. after a constant concentration, $C_{Pl}$, had been maintained in the plasma for 1 hr.

| Age (weeks) | $\frac{C_{Aq}}{C_{Pl}}$ | $\frac{C_{Csf}}{C_{Pl}}$ |
|---|---|---|
| 6 | 0·24 | 0·0017 |
| 9 | 0·145 | 0·003 |
| 11 | 0·115 | — |
| 13 | 0·094 | 0·0019 |
| 17 | 0·090 | 0·0020 |
| 21 | 0·060 | 0·0020 |

obtained, was studied. At successive ages, the rates of penetration of *p*-amino-hippurate into both fluids of one rabbit were examined. The results are shown in Table 6, which gives the values of $C_{Aq}/C_{Pl}$ and $C_{Csf}/C_{Pl}$ after a constant level of *p*-amino-hippurate had been maintained for 60 min in the blood. It will be seen that the value of $C_{Aq}/C_{Pl}$ decreases in a period of 15 weeks by a factor of 4; the blood-c.s.f. barrier, on the other hand, seems already to be established at its adult value at the age of 6 weeks. This finding is consistent with the studies of Flexner (1938) on the steady-state distributions of $Cl^-$ and urea in embryonic and foetal pigs; at term, these were characteristic of the adult animal. Also Stern & Peyrot (1927) found that the blood-c.s.f. barrier to ferrocyanide attained its adult characteristic in the rabbit within a few days of birth.

*Lack of homogeneity*

There is reason to believe that the ventricular fluid equilibrates more rapidly with the plasma than does the subarachnoid fluid, at any rate so far as $^{24}Na$ is concerned (Sweet, Selverstone, Solomon & Bakay, 1949). To test the homogeneity or otherwise of the c.s.f. during the approach to the steady state, three

samples were withdrawn, in immediate succession, from the cisterna magna 30 min after a constant concentration of $^{24}Na$ and ethyl thiourea had been established in the blood; the first two samples had volumes of about 0·25 ml. whilst the third, obtained by continuing the withdrawal until no further fluid could be obtained, had a volume of about 1·0 ml. The mean results of four experiments were as follows, where $r_1$, $r_2$ and $r_3$ are the values of $C_{Csf}/C_{Pl}$ for each sample at the time of withdrawal:

| | $^{24}Na$ | Ethyl thiourea |
|---|---|---|
| $r_1$ | 0·153 | 0·625 |
| $r_2$ | 0·111 | 0·585 |
| $r_3$ | 0·101 | 0·630 |

It will be seen that with the rapidly penetrating substance, ethyl thiourea, the fluid is nearly homogeneous, whilst with $^{24}Na$ the first sample, consisting of fluid that has recently left the ventricles, has about a 50% greater concentration of this isotope than the last, which is probably derived from the more remote regions of the subarachnoid space.

## DISCUSSION

The interpretation of the steady-state distributions described in this paper depends on a knowledge of the kinetics of penetration of the substances concerned. In the light of earlier work (Davson & Matchett, 1953), it may be stated that three major processes govern the concentration, at any time $t$, of a penetrating substance in the aqueous humour. These are: (*a*) Diffusion from plasma into the secretory cells of the ciliary epithelium in accordance with the equation

$$\frac{dC_s}{dt} = k_s (C_{Pl} - C_s), \tag{1}$$

where $C_s$ is the concentration in the cells. These cells eject a fluid, of concentration $C_s$, into the posterior chamber of the eye at a rate given by a flow constant, $k_f$. (*b*) A direct diffusion from the iris into the anterior chamber at a rate given by $k_d (C_{Pl} - C_{Aq})$. (*c*) A loss from the aqueous humour by flow into the canal of Schlemm at a rate given by $k_f C_{Aq}$. The differential equation describing these processes is:

$$\frac{dC_{Aq}}{dt} = k_f C_s + k_d (C_{Pl} - C_{Aq}) - k_f C_{Aq}. \tag{2}$$

Reasons were given for supposing that with relatively rapidly penetrating substances the rate of equilibration of the secretory cells would be large compared with the other processes: in this event the secreted fluid, entering the posterior chamber, would have approximately the same concentration of the solute as that in plasma, i.e. $C_s$ would be approximately equal to $C_{Pl}$. Equation (2) then takes the form

$$\frac{dC_{Aq}}{dt} = k (C_{Pl} - C_{Aq}), \tag{3}$$

which on integration gives

$$\ln\left(1-\frac{C_{Aq}}{C_{Pl}}\right)=-kt.$$

At infinite time a steady state is reached with $C_{Aq}/C_{Pl}$ $(=r)$ equal to unity. When the rate of equilibration of the secretory cells could not be treated as infinite, the solution of equation (2) gave a double exponential expression; this showed that the ratio, $r$, at infinite time must now be less than unity; apart from this, however, its employment was not practicable in the analysis of experimental results, and a more empirical approach was adopted. It was assumed that when the value of $r$ was fairly close to unity, the rate of approach to the steady state would be approximately governed by an equation similar to (3) namely:

$$\frac{dC_{Aq}}{dt}=k_{In}C_{Pl}-k_{Out}C_{Aq},$$

which on integration gives

$$\ln\left(1-\frac{C_{Aq}}{rC_{Pl}}\right)=-k_{Out}t, \tag{4}$$

$r$, the steady-state ratio, being equal to $k_{In}/k_{Out}$. Thus, if it was not practicable to determine $r$ directly, the left-hand function was plotted against time, choosing different values of $r$ until the best straight line was obtained. From the slope of the line, $k_{Out}$ could then be determined. This approach, as pointed out earlier, cannot be completely justified theoretically, but in so far as it provides a numerical parameter, $k_{Out}$ or $k_{In}$, which indicates how rapidly the value of $C_{Aq}/C_{Pl}$ approaches a definite value, $r$, it is of value. To summarize, therefore, if we ignore such complicating factors as losses of material from the aqueous humour to the lens, vitreous body and cornea, as was done in the earlier paper, we may expect, with many substances, to obtain a straight line by plotting $\ln\left(1-\frac{C_{Aq}}{rC_{Pl}}\right)$ against time; with the relatively rapidly penetrating non-electrolytes, $r$ will be equal to unity, i.e. $k_{In}$ will be equal to $k_{Out}$.

To apply the same treatment to the cerebrospinal system we could assume that analogous mechanisms of penetration are present, namely, a penetration into the secretory cells of the choroid plexuses, and carriage into the ventricles with the secreted fluid, associated with a direct diffusion from the blood vessels of the pia and the parenchyma of the central nervous system. The kinetics of equilibration, in this event, should not differ greatly from those observed with the aqueous humour, although the lack of homogeneity in the fluid might be expected to cause deviations from a simple logarithmic relationship between $C_{Csf}/C_{Pl}$ and time. From Figs. 2 and 3 it is seen that rapidly penetrating substances, such as ethyl alcohol and the substituted thioureas, apparently approach a steady-state ratio of unity, whilst less rapidly penetrating substances, such as creatinine, approach a steady-state ratio considerably less

than unity. That in many instances the process of equilibration with plasma follows an approximately first-order equation, moreover, is shown by Fig. 6, in which $\ln\left(1-\frac{C_{Csf}}{rC_{Pl}}\right)$ is plotted against time, $r$ being the steady-state ratio approached at infinite time. The plotted points, in general, fall on straight lines. From the slopes of these lines, therefore, it is possible to derive values of a parameter, $k_{In}$, with dimensions of $t^{-1}$, that adequately indicate the relative rates of achievement of the steady state. An exception is provided by $^{42}K$;

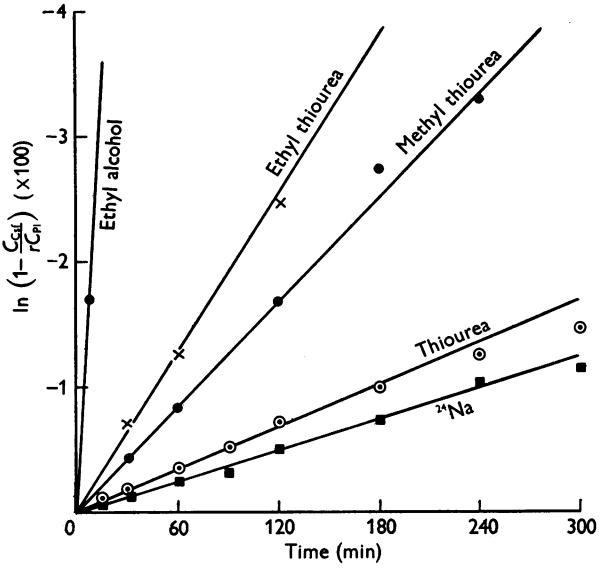


Fig. 6. Penetration of certain substances into the c.s.f.

Ordinates: $\ln\left(1-\frac{C_{Csf}}{rC_{Pl}}\right)$, where $C_{Csf}$ and $C_{Pl}$ are concentrations in c.s.f. and plasma at a given time, and $r$ is the value of $C_{Csf}/C_{Pl}$ at infinite time (taken as unity for the non-electrolytes, and 1·03 for $^{24}Na$)

Abscissae: time in min.

the steady-state distribution ratio is 0·52 (Table 1) and if this value is used to plot $\ln\left(1-\frac{C_{Csf}}{rC_{Pl}}\right)$ against time (not shown in Fig. 6) a serious falling off in slope is found as time increases. This is presumably due to its diffusion from the c.s.f. into the central nervous parenchyma which, with its high average concentration of potassium, will act as a 'drain' for the isotope. To a smaller extent the graphs for $^{24}Na$ and thiourea show a tendency to deviate from linearity at high values of $t$ (Fig. 6); this is most probably due to the absence of homogeneity in the samples, the newly formed fluid mixing only slowly with that distributed throughout the subarachnoid space. In order that

parameters, derived from the slopes of the lines of Fig. 6, may be directly comparable with similar ones obtained from measurements on the aqueous humour, it would have to be shown that the complicating factors in the eye, namely the losses of penetrating material to the lens and vitreous body, were not sufficiently serious to modify the simple exponential relationships established above, nor yet to influence appreciably the values of the steady-state ratios approached during the course of the experimental study. Studies on the lens have shown, however, that uptake by this body is a serious factor which may impose specious steady-state distribution ratios for the penetration of the thioureas. In the case of $^{24}Na$ it would seem that the vitreous body is the dominant factor in determining a specious steady-state ratio of about 0·89, and with $^{42}K$ probably both factors combine to produce a serious deviation from a simple first-order process. (To save space, this deviation has not been demonstrated by a logarithmic plot; it is fairly evident from Fig. 3, however.) Strictly speaking, therefore, a comparison of parameters, of the form $k_{In}$, derived from the slopes of $\ln\left(1-\frac{C_{Aq}}{rC_{Pl}}\right)$ and $\ln\left(1-\frac{C_{Csf}}{rC_{Pl}}\right)$ against time, would be unjustifiable, except possibly in the case of the penetration of ethyl alcohol, which is sufficiently rapid to make losses to the lens and vitreous body negligible. Rather than abstain entirely from quantitative comparisons, however, it would be preferable to make some approximation that would permit of deductions as to relative rates of equilibration that would not be misleading. Fig. 7 shows that the course of equilibration of the aqueous humour with plasma does, with a number of substances, follow a roughly logarithmic course if $\ln\left(1-\frac{C_{Aq}}{r'C_{Pl}}\right)$ is plotted against time; i.e. the course of equilibration apparently follows the simple equation (4), with $r'$ equal to the specious steady-state ratio rather than the true one that would presumably be reached at infinite time. In other words, the rate of approach to the specious steady state may be approximately indicated by $k_{In}$ and $r'$ or $k_{Out}$ and $r'$, where $k_{Out}$ is given by the slopes of the lines in Fig. 7; $k_{Out}$ is greater than $k_{In}$ because of the loss of material to the lens, vitreous body and cornea. Consequently, if any parameter is to be chosen as a measure of the factors favouring the influx of material into the eye, $k_{Out}$ would be preferable to $k_{In}$ since it includes the material that has entered the aqueous humour and passed out into the intra-ocular tissues. With this approximation, one may make a rather closer comparison between the rates of equilibration of the two fluids with plasma than is possible by a mere inspection of Figs. 1–3. From the slopes of the lines in Figs. 6 and 7 the following values for $k_{Out}$ may be derived (Table 7).

With regard to the much more slowly penetrating substances, such as creatinine, sucrose, and *p*-amino-hippurate, it would not be profitable to attempt to compute any simple parameter characterizing their relative rates

of equilibration. We must content ourselves with a simple statement of the order of rates of penetration as deduced from the amounts penetrating in the two fluids in a given time, as follows:

c.s.f.: creatinine > sucrose > *p*-amino-hippurate;

aqueous humour: creatinine > sucrose, *p*-amino-hippurate.

It is reasonable to deduce, however, that the discrepancy in the rates of equilibration between plasma and aqueous humour, on the one hand, and

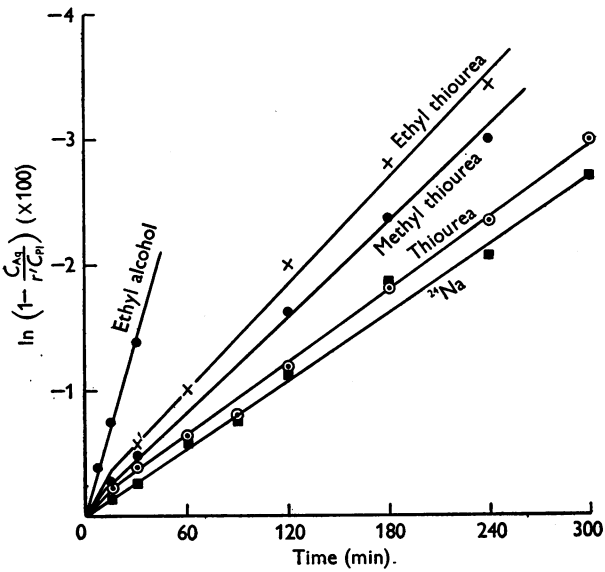


Fig. 7. Penetration of certain substances into the aqueous humour.

Ordinates: $\ln\left(1-\frac{C_{Aq}}{r'C_{Pl}}\right)$ where $C_{Aq}$ and $C_{Pl}$ are concentrations in aqueous humour and plasma at a given time, and $r'$ is the specious steady state approached by the ratio: $C_{Aq}/C_{Pl}$ (taken as 0·85, 0·89, 0·925 and 0·96 for thiourea, $^{24}Na$, methyl thiourea and ethyl thiourea respectively; a true steady-state ratio of unity has been assumed for ethyl alcohol). Abscissae: time in min.

TABLE 7. Values of $k_{Out}$ deduced from the slopes of the lines in Figs. 6 and 7 (a value for $^{82}Br$ is included; its penetration into the aqueous humour has not been plotted in Fig. 7 to avoid overlapping of the graphs)

| Substance | $k_{Out}$ (min$^{-1}$) Aqueous humour | c.s.f. |
|---|---|---|
| Thiourea | 0·00965 | 0·0057 |
| Methyl thiourea | 0·0125 | 0·0135 |
| Ethyl thiourea | 0·015 | 0·021 |
| Propyl thiourea | 0·022 | 0·035 |
| Ethyl alcohol | 0·050 | 0·225 |
| $^{24}Na$ | 0·0090 | 0·0041 |
| $^{82}Br$ | 0·0120 | — |

plasma and c.s.f. on the other, is greater with *p*-amino-hippurate than with creatinine; thus, from Table 3 we can see that with creatinine, after 2 hr, the ratio $C_{Aq}/C_{Pl}$ is some 3 times greater than $C_{Csf}/C_{Pl}$, whilst with *p*-amino-hippurate the ratios differ by a factor of 20.

Reviewing these results on non-electrolytes, we may conclude that, as the rate of equilibration increases, the discrepancy between the aqueous humour and c.s.f. decreases to the point where the rates become about equal (with methyl thiourea); beyond this point the discrepancy changes sign, in the sense that equilibration with c.s.f. is the more rapid process, so that with ethyl alcohol the rate of equilibration of c.s.f. with plasma is some five times greater than the rate for the aqueous humour. The explanation for this trend could be provided by two assumptions: (*a*) that the rate of turn-over of the c.s.f. is less than that of the aqueous humour, and (*b*) that the factors favouring direct diffusion into the c.s.f. are greater than those for the aqueous humour. Thus, with substances that penetrate relatively slowly, such as creatinine and thiourea, there is good reason to believe that direct diffusion into the aqueous humour plays an insignificant role, the great bulk of material being carried into the eye by secretory flow (Davson & Matchett, 1953); if the same consideration were applied to the c.s.f., the rate of equilibration would be smaller with this fluid. As the lipoid solubility of the penetrating substance increased, however, the importance of diffusion would increase and eventually lead to the more rapid equilibration of the c.s.f. The importance of diffusion into the c.s.f. from the nervous tissue is revealed by Table 5 showing the upper and lower limits of the concentration of the penetrating substance in the interstitial fluid; with creatinine the gradient favouring diffusion into the c.s.f. is small; as lipoid solubility increases, the gradient becomes greater and is more rapidly established. With very slowly penetrating molecules, such as *p*-amino-hippurate and sucrose, penetration is presumably through intercellular pores (Davson, 1953); the very large discrepancy in rates of equilibration with these substances could be due to the existence of far fewer pores in the cerebrospinal system, a view consistent with the presence of less protein in the c.s.f. than in the aqueous humour.

*Glucose.* Both the fluids exhibit true steady-state ratios deviating from unity (0·86 and 0·64 in aqueous humour and c.s.f. respectively), due, presumably, to consumption by adjoining tissues. In the absence of complete curves describing the penetration of glucose, an assessment of values for $k_{Out}$ must be very provisional; the difference between the values for the two fluids revealed, however, is sufficiently great to justify the belief that equilibration with the aqueous humour is by far the more rapid process. Thus $k_{Out}$ for the aqueous humour will be given by

$$\frac{1}{t}\ln\frac{(rC_{Pl}-C_{Aq^1})}{(rC_{Pl}-C_{Aq^2})},$$

substituting the values shown in the 'Results' gives 0·0155 $min^{-1}$; a similar calculation gives 0·0087 $min^{-1}$ for the c.s.f. Hence it would appear that equilibration between plasma and c.s.f. is the slower process.

*Penetration of ions.* In the aqueous humour it would appear from Fig. 3 and Table 3 that the rates of equilibration of ions decrease in the order

$$Br^- > CNS^- > K^+ > Na^+ > I^-,$$

the position of $K^+$ being doubtful, however, owing to the uptake by the lens. So far as the c.s.f. is concerned, it is not easy to establish a similar series with any degree of confidence. Thus the losses of $^{42}K$ to the nervous tissue are apparently so large that it would be unreasonable to make any quantitative comparison between the kinetics of its penetration and those of $^{24}Na$, beyond the statement that penetration of $^{42}K$ may be slower, since even in the first 30 min the value of $C_{Csf}/C_{Pl}$ is some 33% less than with $^{24}Na$. Again, the behaviour of $^{82}Br$ is anomalous, as the following considerations will show. The true steady-state ratio for this ion is 0·715 (Table 1); its initial rate of equilibration with plasma, however, is at least equal to, if not greater than, that of $^{24}Na$, which gives a steady-state ratio of just over unity. Consequently, $^{82}Br$ penetrates at an initial rate that is inconsistent with its final steady state, if we view the process on simple kinetic grounds. An explanation that would fit the facts is that $^{82}Br$ is incorporated into a complex in the nervous tissue, but that this complex diffuses away into the blood stream to be eventually excreted. This mechanism would constitute a steady 'drain' by which $^{82}Br$ diffused out into the nervous tissue without accumulating there and would lead to a steady state of less than unity that would last indefinitely. It would also provide a possible explanation for the finding of Weir (1942) that the value of the distribution ratio approached unity with very large concentrations of bromide in the blood (90–95 mM); thus it could be assumed that the chemical mechanisms for incorporating bromide into a complex became saturated at very high concentrations of the substrate. The steady-state ratio for $^{131}I$ is very low and variable—0·004–0·04—and the rate of equilibration with plasma, as indicated by the value of $C_{Csf}/C_{Pl}$ at 120 min, is low; equilibration between plasma and aqueous humour is relatively slow, so that by analogy with the behaviour of non-electrolytes we can expect a slower rate of penetration of $^{131}I$ into the c.s.f. than into the aqueous humour, but not such a large discrepancy; it may well be that a similar or greater loss to the nervous tissue is operative here, so that the kinetics are obscured. Some unpublished studies on the passage of $^{131}I$ and $^{24}Na$ out of the subarachnoid space, after injection into the cisterna magna, support this view. $^{131}I$ disappears from the c.s.f. about twice as rapidly as $^{24}Na$, so that its passage into the nervous tissue may be some three times as rapid as that of $^{24}Na$ if allowance is made for the removal of both ions by drainage at the same rate into the venous sinuses. This could be the result of

a conversion of iodide into some other substance by the nervous tissue. Again, an inspection of the studies of Wallace & Brodie (1940) on iodide and thiocyanate indicates an anomalous kinetic behaviour; thus, after a single injection of iodide, the ratio $C_{Csf}/C_{Pl}$ steadily rose, owing to the rise in iodide concentration in the c.s.f. and the fall in the plasma concentration. At 24 hr the ratio was 0·14, but 5 hr later, although the concentration in the plasma had only dropped by about 5%, the ratio had fallen to about 0·02, due to a precipitate fall in the concentration of iodide in the c.s.f.

So far as earlier quantitative studies on ions are concerned, the results described here for $^{24}Na$ agree with those of Wang (1948) on the dog, in that he found that this isotope penetrated into the aqueous humour more rapidly than into the c.s.f. The absolute rates, indicated by values of $k_{In}$, were very much higher (0·025 and 0·0073 $min^{-1}$ respectively), so that there is an obvious species difference in turn-over rates for this ion. Greenberg, Aird, Boelter, Campbell, Cohn & Murayama (1943) studied the penetration of ion isotopes into the c.s.f. of the dog; their experimental procedure involved a preliminary more or less complete emptying of the cerebrospinal system; the isotope was then injected intravenously as a single dose, and the concentrations in successive samples of the continuously dripping fluid were determined. According to these workers, $^{42}K$ penetrates more rapidly than $^{24}Na$.

For both c.s.f. and aqueous humour the steady-state concentration of sodium is greater than that required by a Donnan distribution; the excess of sodium is always greater in the c.s.f. and, in the rabbit, is more than balanced electrostatically by an excess of chloride; in the aqueous humour, by contrast, the state of affairs regarding the excess of chloride varies from species to species. The feature of the ionic distribution that seems to be shared by both fluids in all species examined, therefore, is the excess of sodium, and it is tempting to conclude that the primary process in the formation of both fluids is an active transport of this ion from the plasma to the ventricle or posterior chamber. The transfer of water and the remaining constituents would then follow in accordance with the requirements of osmotic and diffusion equilibria; the constant drainage of the fluid away from the site of its formation would prevent the establishment of true equilibria in many cases, and the steady-state distributions actually achieved would be determined, in the first place, by the permeability characteristics of the lining membranes of the cavities. Thus, if diffusion of potassium were relatively slow in the cerebrospinal system, we might explain the low steady-state ratio on these grounds. In certain cases, of course, specific secretory activity may well operate (in addition to that taking place with sodium) as, for example, with ascorbic acid, the magnesium ion (McCance & Watchorn, 1931), and the chloride ion.

In conclusion, the bearing of the results described here on an earlier study may be briefly ndicated. It was shown (Davson & Matchett, 1953) that the steady-state distribution ratio to

which thiourea approached, after long periods of intravenous infusion, was 0·95; this work was carried out on young animals. In the large adult animal the rate of equilibration is less and consequently the smaller ratio found here, namely 0·85, is not surprising. In the earlier paper this steady-state ratio was taken as a true one, appropriate to infinite time, whereas the studies of the lens described here make it most probable that the true ratio, for both young and old animals, is indeed unity. Thus, from the observed rate of uptake by the lens in large animals, and on the assumption that all the material entering the lens comes directly from the aqueous humour, it is possible to predict a temporary steady-state ratio of about 0·85. If the true steady-state ratio for thiourea is indeed unity, then the arguments developed in the earlier paper are enforced. The demonstration, moreover, that thiourea penetrates into the lens at a rate independent of the pupillary area shows that the uptake occurs in the posterior chamber—in other words that non-electrolytes are certainly not excluded from the primary secretion from the ciliary body.

SUMMARY

1. The distributions of sodium, potassium, chloride, bromide, iodide, glucose and ascorbic acid between blood plasma and aqueous humour, on the one hand, and between plasma and cerebrospinal fluid (c.s.f.) on the other, have been determined in rabbits. Similar distributions for sodium and chloride have been determined in the horse, dog, cat and guinea-pig. The electrical conductivity and depression of freezing-point of aqueous humour and c.s.f. have also been compared.

2. The course of penetration of a number of non-electrolytes and ions into the aqueous humour and c.s.f. of the rabbit has been studied. The results have revealed an essential similarity between the blood-aqueous and blood-c.s.f. barriers, such differences as are observed being explicable on the assumption that the percentage rate of renewal of the aqueous humour is more rapid than that of the c.s.f., but that a direct diffusion from the blood vessels of the subarachnoid space and nervous tissue into the c.s.f. is a more significant factor than direct diffusion from the vessels of the iris into the anterior chamber of the eye.

3. With a number of substances the blood-c.s.f. and blood-brain barriers were studied simultaneously. $^{24}Na$ distributes itself between plasma and c.s.f. at about the same rate as between plasma and the extracellular fluid of the brain; with all the other substances studied, namely creatinine, thiourea and its alkyl-substituted derivatives, and ethyl alcohol, distribution between plasma and brain extracellular fluid is the more rapid process.

4. The kinetics of the process of penetration of various substances into the aqueous humour and c.s.f. have been briefly analysed; this has required a knowledge of the kinetics of uptake of material by the lens and vitreous body, and it has been shown that some apparent anomalies in the kinetics, as the steady-state distribution between plasma and aqueous humour is approached, may be accounted for by uptake by these intra-ocular bodies from the aqueous humour. The kinetics of penetration of bromide into the c.s.f. exhibit an anomaly which may be due to a detoxication mechanism in the nervous tissue; it is

probable that a similar mechanism operates with respect to iodide and thiocyanate.

5. The blood-aqueous barrier to *p*-amino-hippurate becomes steadily more efficient as the animal increases in age from 6 weeks to 5 months; the blood-c.s.f. barrier to this substance has acquired its adult characteristics at 6 weeks, if not before.

6. The steady-state distribution ratios are discussed in the light of the kinetic studies; it is suggested that, in both ocular and cerebrospinal systems, the primary process in the formation of the fluids is an active transport of sodium from plasma into the posterior chamber or ventricle.

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