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THE TRANSPORT OF UREA, CREATININE AND CERTAIN MONOSACCHARIDES BETWEEN BLOOD AND FLUID PERFUSING THE CEREBRAL VENTRICULAR SYSTEM OF RABBITS

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The development of the technique of ventriculo-cisternal perfusion has provided a useful method for the quantitative study of the transport of substances between blood, brain and cerebrospinal fluid. By its use in the goat Pappenheimer, Heisey & Jordan (1961) first demonstrated the active transport of diodrast and phenol red from cerebrospinal fluid to blood.

The technique, which was adapted to the rabbit by Pollay & Davson (1963), has been used here to investigate the mechanisms of exchange of urea, creatinine and certain monosaccharides, between blood, brain and cerebrospinal fluid. The results have permitted the conclusion that simple non-carrier-mediated diffusional processes are concerned with the exchange of urea and creatinine, whilst a carrier-mediated mechanism seems to be involved in the exchanges of the sugars.

METHODS

The experiments were carried out on albino rabbits, weighing between 2·0 and 3·5 kg. Anaesthesia was induced with intravenous sodium pentobarbitone (Nembutal, Abbott Laboratories) 40 mg/kg, and open drop ether. Anaesthesia was maintained with intravenous injections of Nembutal given as necessary.

Ventriculo-cisternal perfusion. Cannulae were inserted into one lateral ventricle and into the cisterna magna as described earlier (Pollay & Davson, 1963). Perfusion of an artificial cerebrospinal fluid containing (mM): NaCl 154, KCl 2·8, $CaCl_2$ 1·1, $MgSO_4$ 0·8 and inulin (80 mg/100 ml.) was established by driving a syringe with the variable-speed injection machine of Davson & Purvis (1952). The proportions of the cations are those found in the cerebrospinal fluid of rabbits. Collection of fluid from the cisterna magna was carried out under a controlled negative pressure of 15 cm H_2O by the sampling device described by Davson, Pollay & Purvis (1962). The rate of perfusion was 34 μl./min.

In certain experiments two inflow cannulae were placed in the rabbit, one in each lateral ventricle. Each cannula was connected to a separate variable-speed injection machine and manometer. The technique of placing for each cannula was exactly similar to that for the single inflow cannula in one lateral ventricle. The rate of flow in these experiments was 34 μl./min into each lateral ventricle, the total inflow being thus 68 μl./min.

In order to study the exit of various non-electrolytes from the perfusion fluid, solutions

were perfused containing ^{14}C-urea, ^{14}C-D-glucose (uniformly labelled), and inactive D-creatinine, xylose and D-fructose. These solutes were perfused in separate experiments at a 'low' concentration and at a 'high' concentration, except in the case of D-fructose which was only perfused at the low concentration. The 'low' concentration of creatinine, D-xylose and D-fructose in the inflow solution was 1·7 mM. The low concentrations of D-glucose and urea were 0·1 mM, though, of course, the outflow concentrations were very much higher, urea and glucose having entered the perfusion fluid from the animal; the average outflow concentrations were 2·2 and 3·4 mM respectively. The 'high' concentration for all solutes was 34 mM. ^{14}C-urea and ^{14}C-D-glucose were present in both 'high' and 'low'-concentration solutions at 10 μc/100 ml. The total osmolarity of the electrolytes in the 34 mM solutions of non-electrolytes was reduced to 95 % of that in the low concentration solutions.

The entry of urea and glucose into the perfusion fluid, the infusion being begun when brain was presumed to be in equilibrium with blood with respect to these solutes, was studied in the following manner. Fluid, containing no urea or glucose, was perfused through the ventricular system of animals that had received no preliminary intravenous treatment. The effluent was sampled and analysed for total urea and glucose by the chemical methods listed below. Similar results for creatinine were obtained in the following manner. The kidneys were removed from four animals under Nembutal and ether anaesthesia. 25 ml. of 4 % creatinine solution was injected slowly into an ear vein and the animals were allowed to recover consciousness. 24 hr later a ventriculo-cisternal perfusion was carried out in the normal manner. Four other animals were treated in this way, but instead of a perfusion being made after 24 hr, the animals were re-anaesthetized and the cerebral hemispheres were removed and weighed. Each hemisphere was homogenized with 10 ml. of 2·5 % sodium tungstate and 10 ml. of N/5 sulphuric acid. After centrifugation, creatinine in a specimen of the supernatant was estimated as the alkaline picrate.

In order to study the entry of solutes into the fluid immediately after the blood level had been raised and kept constant, i.e. while the concentration of the solute in brain was increasing, the following experiments were performed. Perfusions were set up in the normal manner. After the fluid had been flowing for 5 min, the blood level of the substance, ^{14}C-urea, creatinine, D-xylose or D-fructose, was raised by intravenous injection and maintained constant by the technique of Davson & Purvis (1952). The average blood concentrations of the various solutes were (mM): creatinine 4·5, xylose 6·5, fructose 2·0 and ^{14}C-urea, tracer concentration only.

Analytical. Creatinine was determined as the alkaline picrate; inulin and fructose by the method of Hubbard & Loomis (1942); urea by that of Conway (1950); glucose by that of Huggett & Nixon (1957); and xylose by the method of Mejbaum (1939). ^{14}C-urea and ^{14}C-glucose were counted in a windowless gas-flow Geiger–Müller counter, the samples having been plated on planchettes, covered with lens paper and dried (Calvin, Heidelberger, Reid, Tolbert & Yankwich, 1949). Specimens from the experiments, in which ^{14}C-urea was maintained at constant concentration in the blood, were counted in a liquid scintillation counter. Corrections for background, decay, dead time of counter and self-absorption were made when necessary.

When artificial cerebrospinal fluid containing no inulin was perfused, the effluent gave a small but definite blank value when analysed for inulin or fructose by the method of Hubbard & Loomis (1942). The blank value can be wholly accounted for by the glucose present in the effluent. It was allowed for in the calculation of cerebrospinal fluid production by the dilution of inulin, and in the studies of the exit of fructose from, and entry of fructose into, the fluid.

Theoretical. If the perfusion fluid contains a substance that is neither absorbed from nor added to it during its passage through the ventricular system, the addition of fluid may be derived from the dilution of the fluid with respect to this substance. Thus, if F_{in} ml./min

is the rate of perfusion and F_{csf} ml./min is the rate of addition of fluid within the ventricular system,

$$F_{csf} = \frac{C'_{in} - C'_{out}}{C'_{out}} \times F_{in}, \tag{1}$$

where C'_{in} and C'_{out} represent the concentrations (mg/ml) of this substance, e.g. inulin, in perfusion fluid and cisternal outflow respectively, after a steady state has been attained.

If the perfusion fluid contains a solute that leaves the fluid during its passage through the ventricular system, then the rate of absorption, or flux N, (mg/min) will be given by

$$N = F_{in}(C_{in} - C_{out}) - F_{csf}C_{out}, \tag{2}$$

where C_{in} and C_{out} are the concentrations of the solute (mg/ml.$^{-1}$) perfusion fluid and cisternal outflow. If the solute is added to the perfusion fluid, the sign of the evaluated N will be negative instead of positive. By substitution from (1), this equation may be rewritten

$$N = F_{in}\left(C_{in} - C_{out}\frac{C'_{in}}{C'_{out}}\right). \tag{3}$$

The flux N may be converted into a transfer constant, K, by dividing it by the concentration difference between perfusion fluid $(C_{in}+C_{out})/2$ and blood plasma (C_{pl}). (The arithmetical mean of C_{in} and C_{out} is used, although there must be doubt as to the real mean concentration in the perfusion fluid during its passage through the ventricular system. Probably most alteration in concentrations takes place in the perfused lateral ventricle before cerebrospinal fluid from the other lateral ventricle enters the stream in the third ventricle.) Thus

$$K = N/\{(C_{in}+C_{out})/2 - C_{pl}\}. \tag{4}$$

K_{out} or K_{in} will be used to refer to the constant derived in the above manner, when the experiments have been concerned with absorption from the perfusion fluid or addition to the fluid respectively. The units of K, as derived, are ml./min. K_{out} is related to the k_{out} of Davson & Pollay (1963) by the formula

$$k_{out} = K_{out}/V, \tag{5}$$

where V is the average volume of cerebrospinal fluid in the rabbit, found to be 1·83 ml.

If a solute such as urea diffuses between blood and perfusion fluid, and is also transported unidirectionally into the perfusion fluid in the newly formed cerebrospinal fluid, such that the concentration in this newly formed fluid, C_{csf}, is given by

$$C_{csf} = rC_{pl}, \tag{6}$$

where r is a constant, then, when the system is in a steady state, the following relation will hold

$$rC_{pl}F_{csf} + C_pK_x = \frac{C_{out}}{2}.K_x + C_{out}(F_{in} + F_{csf}); \tag{7}$$

whence

$$r = \frac{C_{out}}{C_{pl}} \times \frac{K_{out}/2 + F_{in} + F_{csf}}{F_{csf}} - \frac{K_{out}}{F_{csf}}. \tag{8}$$

Here K_x is a transfer constant (ml./min) relating to the permeability of the total barrier to diffusion between blood and perfusion fluid. Since diffusion occurs largely across nervous tissue, the system is not a simple two-compartment one and this constant K_x is only meaningful when the system is in a steady state. If a fluid containing ^{14}C-urea is perfused through the ventriculo-cisternal system, K_x will be given by K_{out} for this substance computed from equation (4). (In one experiment in which urea solution was perfused at the

high concentration, the K_{out} for total urea was found to be 0·0073. The corresponding value for ^{14}C-urea, determined simultaneously, was 0·0103. This indicates that the clearance of total urea from the fluid is not greater than the clearance of tracer due to a long pore or other effect. In view of this and of the findings of Kleeman, Davson & Levin (1962), we have felt justified in assuming that diffusion of the tracer follows the same kinetics as that of urea across a concentration gradient in our system. That the clearance of the tracer is in fact greater by 0·0030 ml./min is compatible with the assumption implicit in equations (7) and (8) that some urea enters the ventricles unidirectionally with the newly formed cerebrospinal fluid, i.e. the net transport of total urea out of the fluid is diminished in comparison with that of ^{14}C-urea by the urea in the new cerebrospinal fluid.)

Results from preparations in which only one lateral ventricle was perfused have not been substituted in equation (8). These preparations would not be completely symmetrical with respect to diffusion of urea in and out, as the unperfused lateral ventricle would be available for diffusion of urea into the fluid, but probably not for the diffusion of ^{14}C-urea out. This difficulty is not present when both lateral ventricles are perfused. r, calculated from equation (8) will be valid, if the steady state has not been quite reached, because of the symmetry of diffusion in both directions.

Rate of flow. It was found that the addition of fluid to the perfusion flow, F_{csf}, estimated by weighing the specimens of effluent after a steady state had been achieved, was slightly less than the value of F_{csf}, calculated from the dilution of inulin (equation 1). A negative pressure of -15 cm H_2O should be enough to prevent any fluid escaping through the arachnoid villi (Welch & Friedman, 1960), and the difference is attributable to the passage of a small quantity of inulin into brain tissue (Rall, Oppelt & Patlak, 1962). In calculations involving F_{csf} the value obtained from the dilution of inulin has been used, but it has first been multiplied by 0·96 to allow for the above loss. When constants K_{out} have been calculated for D-fructose, F_{csf} determined gravimetrically has been used.

RESULTS

Exit of substances from the perfusion fluid

In the experiments illustrated by Fig. 1 a fluid containing either ^{14}C-urea, creatinine or ^{14}C-glucose at the low concentration was perfused through the ventriculo-cisternal system. As cerebrospinal fluid is replaced by perfusion fluid in the ventricular system, the concentrations of all substances rise in the effluent, and after 60 min perfusion are slowly approaching a steady state. Creatinine, ^{14}C-urea and ^{14}C-glucose leave the system at rates that increase in this order. Since the concentrations of the various substances in the effluent are only changing slowly after 60 min, it was considered that the mean values of C_{out} at 80, 100 and 120 min could be taken as approximations to steady-state concentrations. Transfer constants K_{out} were computed on the basis of equation (4), these concentrations being used. These constants, together with values for xylose and fructose, are listed in Table 1. The values of K_{out} for ^{14}C-urea and creatinine at the low concentration, 0·0090 and 0·0049 respectively, are not changed significantly at the high concentration, when the values found were 0·0091 and 0·0059. At the low concentration the constants for ^{14}C-glucose and xylose were 0·0191 and 0·0147 respectively. The constants for fructose and for ^{14}C-fructose were much less than those for the other two

monosaccharides investigated, being 0·0055 and 0·0057 respectively. The constants K_{out} for ^{14}C-glucose and for xylose at the high concentration were significantly depressed below the values for the low concentration of

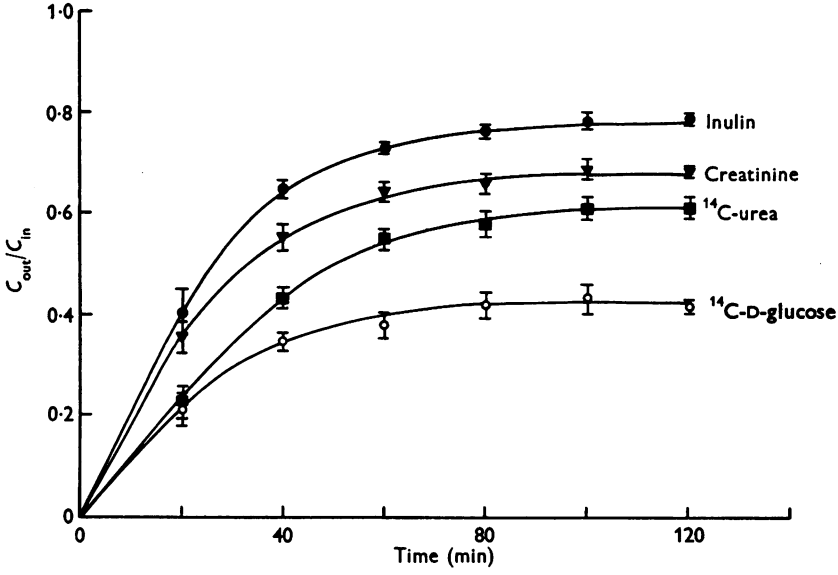

Fig. 1. (Concn. in outflow):(concn. in inflow) for inulin, 80 mg/100 ml., and creatinine, ^{14}C-urea and ^{14}C-glucose at low concentration in fluid being perfused from lateral ventricle to cisterna magna of the rabbit at 34 μl./min.

TABLE 1. Constants K_{out} (ml./min^{-1} × 10^3) for the clearance of solutes being perfused from lateral ventricle to cisterna magna of the rabbit. Average number of animals in each group is 4. Limits ± are standard errors of means

Solute	At 'low' concn.	At 'high' concn. (34 mM)	At low concn. plus other sugar	At low concn. plus DNP (0·05 mM)
^{14}C-urea	9·0 ± 0·7	9·1 ± 0·6	—	—
Creatinine	4·9 ± 0·4	5·9 ± 0·7	—	—
^{14}C-D-glucose	19·1 ± 1·0	10·1 ± 1·1	12·7 ± 0·7 (plus D-xylose 34 mM)	21·0 ± 3·4
D-xylose	14·7 ± 1·2	9·9 ± 0·7	—	—
D-fructose	5·5 ± 0·3	—	—	—
^{14}C-D-fructose	5·7 ± 0·5	—	4·1 ± 0·3 (plus D-glucose 34 mM)	—

the relevant sugar. Further, the presence of xylose at the high concentration, 34 mM, caused a significant depression of the constant for ^{14}C-glucose, whilst glucose at the high concentration lowered the constant for ^{14}C-fructose. Dinitrophenol (0·05 mM) in the perfusion fluid was without effect on the exit of the ^{14}C-glucose at the low concentration. In one experiment, in which a glucose solution was perfused at the high concentration, the K_{out} for total glucose was 0·0047. The corresponding value for ^{14}C-glucose, determined simultaneously, was 0·0106.

Entry of substances into perfusion fluid after the brain is in equilibrium with blood

Figure 2 illustrates the change in concentration of the emerging fluid, when a perfusion is carried out with a solution that contains none, or only trace quantities, of the solute being investigated. Thus the substance enters from the surrounding nervous tissue and from the choroid plexuses.

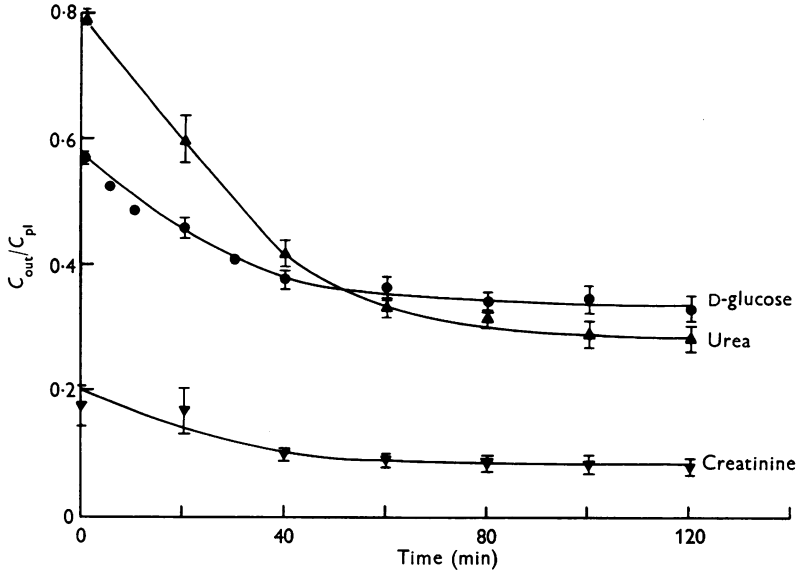

Fig. 2. (Concn. in outflow):(concn. in plasma water) when fluid, containing no urea, glucose or creatinine, was perfused from lateral ventricle to cisterna magna of the rabbit at 34 μl./min. Urea and glucose are derived from the normal body content of these substances. Creatinine values are from rabbits which had been nephrectomized and injected intravenously with 25 ml. of 4% creatinine 24 hr before.

The urea and glucose results were obtained from normal animals into which no exogenous material was infused. The creatinine results were obtained from animals which had been nephrectomized 24 hr before and injected intravenously with 25 ml. of 4% creatinine. The ratios $C_{out}:C_{pl}$ start high and, as perfusion fluid replaces the original cerebrospinal fluid, fall at a decreasing rate towards equilibrium values. After 60 min perfusion the rates of entry into the perfusion fluid decrease in the order glucose, urea, creatinine. It is of interest that the curves for glucose and urea cross at about 50 min. The normal cerebrospinal fluid:plasma ratio for glucose (0·57) is less than that for urea (0·78), but at 120 min the perfusion fluid:plasma ratio is greater for glucose than for urea.

Constants K_{in} have been calculated by substitution in equation (4) for creatinine, urea and glucose from the means of their C_{out} values at 80, 100, 120 min, and are recorded in Table 2. Alongside the K_{in} values for urea and glucose are given the K_{out} values for ^{14}C-urea and ^{14}C-glucose determined simultaneously in the same animals with tracer quantities of the compounds. The K_{in} for urea is greater by 0·0072 than the K_{out} for labelled urea. In the case of glucose the difference is less and in the other direction, −0·0032.

Steady-state distributions. Four animals were nephrectomized and injected with creatinine in the above manner. After 24 hr cerebrospinal

TABLE 2. Constants K_{in} (ml./min × 10^3) for the entry of urea, glucose and creatinine into fluid being perfused from the lateral ventricle to cisterna magna. Urea and glucose in the fluid are from the rabbit's normal body content of these substances. Creatinine values are from rabbits which had been nephrectomized and injected with 25 ml. of 4% creatinine, 24 hr before. K_{out} values determined simultaneously in the same animals with traces of ^{14}C-urea and ^{14}C-D-glucose are given for comparison. Average number of animals in each group is 4. Limits ± are standard errors of means

Solute	K_{in} (total solute)	K_{out} (radioactive tracer)	$K_{in} - K_{out}$
Urea	16·2 ± 1·2	9·0 ± 0·7	7·2 ± 0·6
D-glucose	17·2 ± 0·7	20·4 ± 1·2	−3·2 ± 1·1
Creatinine	3·8 ± 0·4	—	—

fluid was sampled and the brains were removed without a perfusion being made. The ratios (concn. in brain H_2O):(concn. in plasma H_2O) and (concn. in c.s.f.):(concn. in plasma H_2O) are well below unity, being respectively 0·33 ± 0·03 and 0·17 ± 0·02. The ratio for cerebrospinal fluid was significantly less than that for brain:water.

Entry of substances into the perfusion fluid immediately after the blood concentration has been raised and kept constant. In Fig. 3, C_{out}/C_{pl} has again been plotted against time, but in this case immediately after the blood concentration of creatinine, ^{14}C-urea, xylose and fructose have been raised and maintained constant by intravenous infusion. The concentrations of creatinine and ^{14}C-urea in the effluent rise fairly rapidly as mixing is taking place over the first 60 min, but a slow rise continues after this. At 110 min the ratios C_{out}/C_{pl}, 0·045 for creatinine and 0·14 for ^{14}C-urea, are far from the values at this time determined in the experiments recorded in Fig. 2, 0·084 for creatinine and 0·290 for urea, i.e. when the steady state is approached from the other direction. Although it has not been possible to study the entry of glucose under these conditions, the fact that xylose in the effluent reached a ratio of 0·33 at 110 min suggests that this sugar equilibrates much more rapidly.

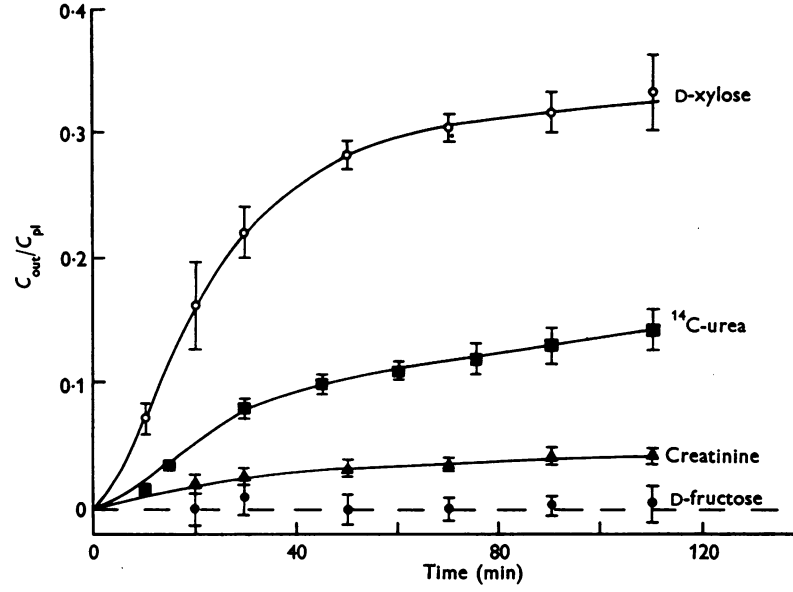

Fig. 3. (Concn. in outflow):(concn. in plasma water) for fluid being perfused from lateral ventricle to cisterna magna of the rabbit at 34 μl./min. The blood concentration of xylose, ^{14}C-urea, creatinine or fructose had been raised at the onset of the perfusion and maintained constant for 2 hr.

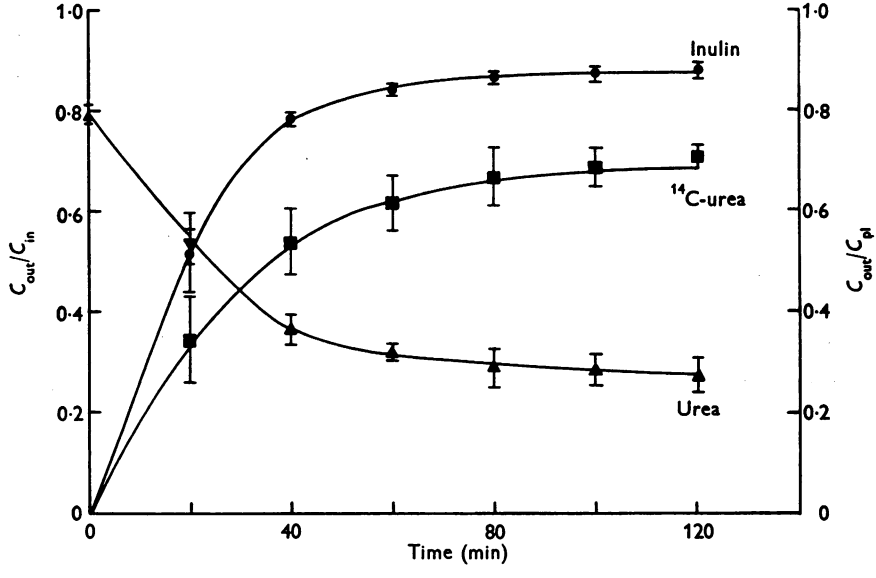

Fig. 4. (Concn. in outflow):(concn. in inflow) for inulin 80 mg/100 ml., and ^{14}C-urea, tracer concentration, in fluid being perfused from both lateral ventricles to cisterna magna of the rabbit at a rate of 34 μl./min into each lateral ventricle. (Concn. in outflow):(concn. in plasma water) for total urea is given for comparison.

Bilateral perfusion of fluid containing tracer quantities of ^{14}C-urea

In Fig. 4 C_{out}/C_{in} for ^{14}C-urea and C_{out}/C_{pl} for total urea have been plotted against time for animals with cannulae perfusing both lateral ventricles with fluid containing ^{14}C-urea, in tracer quantities only. In Table 3 are given the mean of the K_{out} values for ^{14}C-urea, K_{in} values for total urea, their differences, and the ratios of urea in newly formed cerebrospinal fluid to urea in plasma, derived from equation (8). The K_{in} and K_{out} values for these bilateral perfusion 0·0249 and 0·0174 are substantially higher than the corresponding values for unilateral perfusions, the latter

TABLE 3. Constant K_{in} for total urea and constant K_{out} for ^{14}C-urea (ml./min × 10^3) determined from four rabbits, having inflow cannulae in both lateral ventricles introducing fluid containing only tracer quantities of ^{14}C-urea. The c.s.f. production and the computed concentration of urea in the primarily secreted c.s.f., relative to plasma, are also given. Limits are standard errors of means

K_{in}(urea)	K_{out}(^{14}C-urea)	$K_{in} - K_{out}$	c.s.f. production (ml./min × 10^3)	r for newly formed c.s.f.
24·9 ± 2·7	17·4 ± 1·9	7·4 ± 1·2	10·1 ± 1·4	0·60 ± 0·04

being almost twice as great. At the end of 2 hr perfusion the concentration of ^{14}C-urea in the water of the cerebral hemispheres as a whole was 0·34 ± 0·02 of the concentration of ^{14}C-urea in the effluent from the cisterna magna at this stage. This indicates that 40–50 % of all the ^{14}C-urea lost from the perfusion fluid is recoverable from the cerebral hemispheres.

DISCUSSION

All the substances added to the perfusion fluid were diluted to a greater extent than inulin so that they must have left the ventricular system by routes other than the non-specific drainage channel through the arachnoid villi that is responsible for the escape of inulin in the intact animal (Davson, Kleeman & Levin, 1962). The possible routes by which this happened are a direct diffusion through the epithelium of the choroid plexuses and into their capillaries, or diffusion into the surrounding nervous tissue and secondarily from there into the capillaries of this tissue. Urea, glucose and xylose entered the fluid from blood at rates faster than would be accounted for by their entering the ventricles in the newly formed cerebrospinal fluid at the same concentration as in plasma. Thus the value of K_{in} for a substance entering in this manner only could not be greater than the cerebrospinal fluid production in ml./min, mean value 0·0113, whilst the actual values for urea, glucose and xylose were 0·0162, 0·0172 (Table 2) and 0·0172 computed from C_{out}/C_{pl} values at 70, 90, 110 min

(Fig. 3). Consequently the measurements of both outward escape and inward penetration indicate that exchanges in addition to those concerned in the secretory process take place. Transport out of the fluid by diffusion into the choriod plexuses would involve diffusion against a flow of fluid, and the present results with urea, the studies of Davson & Pollay (1963) with ^{24}Na and most other quantitative work on blood-cerebrospinal fluid exchanges suggest that the route through nervous tissue is the most important one for diffusion (Olsen & Rudolph (1955); Mayer, Maickel & Brodie (1960)).

We may now consider some of the findings in detail.

Urea. The experiments in which ^{14}C-urea was perfused in tracer quantities, and in a solution containing 34 mM urea, support uncomplicated diffusion as the mechanism by which urea is leaving the fluid, the mean transfer constants being very similar for the two urea concentrations. It is possible that a carrier is involved which is functioning at well below half saturation, even in the high concentration of urea (Wilbrandt & Rosenberg, 1961), but there is no indication that this is so. Since K_{out} for ^{14}C-urea, determined from the bilateral perfusion experiments, was nearly twice that from the unilateral perfusions, most diffusion of this substance out of the fluid must occur from the lateral ventricles. In the unilateral experiments little or none of the artificial cerebrospinal fluid can have entered the unperfused lateral ventricle by retrograde flow from the third ventricle.

Transfer constants K_{in} for urea (Table 2) are invariably higher than the corresponding K_{out} for ^{14}C-urea. If the transport of traces of ^{14}C-urea follows the same kinetics as the transport of total urea across a concentration gradient, and experiments in which a fluid containing a high concentration of urea is perfused indicate that this is so, then transport of urea into cerebrospinal fluid must be favoured over transport out in some manner. The simplest hypothesis to account for this difference is to suppose that some urea is transported unidirectionally across the choroidal epithelium, possibly by solvent drag along with the newly formed cerebrospinal fluid (Andersen & Ussing, 1957). Since transfer constants are known for transport in and out, the concentration of urea in the new fluid can be derived from equation (8). It was not considered valid to make this calculation with figures determined from experiments in which only one of the lateral ventricles was perfused. The preparation would not be completely symmetrical with respect to diffusion of urea in and out, as the unperfused lateral ventricle would be available for the diffusion of urea into the fluid, but probably not for the diffusion of ^{14}C-urea out. Hence the bilateral perfusions were performed. A similar picture emerged, K_{in} always being higher than K_{out} and the difference being of similar size,

0·0074. Substitution in equation (8) yields a value for r of 0·60. In other words, the newly formed fluid as it enters the ventricle would have a concentration some 60 % of that in blood. The actual concentration in fluid drawn from the cisterna magna, which is a mixture of fluids in the ventricles and subarachnoid space with the latter predominating, is 78 % of that in plasma (Kleeman *et al.* 1962). Diffusion between nervous tissue and fluid as it flows through the ventricles and subarachnoid spaces could well raise the ratio from 0·60 to 0·78.

Recent observations on specimens of ventricular and lumbar cerebrospinal fluid sampled simultaneously from human subjects gave ratios of 0·63 and 0·81 respectively (Bradbury, Stubbs, Hughes & Parker, 1963). So it is reasonable to suppose that the original fluid secreted is low in urea and its concentration is increased by diffusion as the fluid passes down the neural axis. Urea is also transported at a slower rate than water in other systems where there is net transport of fluid. Thus Kinsey, Reddy & Skrentny (1960) computed from kinetic studies of the transport of ^{14}C-urea into the eye of the rabbit that the concentration of urea in the newly secreted aqueous humour must be about 50 % of that in plasma. Fisher (1955) showed that, when water is being absorbed from the lumen of an isolated loop of small intestine, urea dissolved in the water is entrained with the stream, and the concentration in the flowing fluid is about 90 % of that in the luminal fluid.

The above evaluation has involved no assumptions as to whether diffusion of urea between fluid and blood occurs directly or across ependyma and nervous tissue. When the cerebral hemispheres are analysed for radioactivity after bilateral perfusion with fluid containing ^{14}C-urea, the counts per millilitre brain-water (no attempt was made at localization) averaged 0·34 of the counts per millilitre in the effluent from the cisterna magna. This represents 40–50 % of the ^{14}C-urea lost from the fluid during its passage through the system. Thus most of the urea lost from the perfusion fluid must go through brain on its route to blood. Because of the delay in passing from brain to blood across the blood–brain barrier, much of the ^{14}C-urea accumulates in brain. This finding is simply an expression of the existence of a blood–brain barrier in respect to urea, i.e. the presence of a considerable diffusion barrier between the capillaries of the nervous tissue and the extracellular space of this tissue. This is further revealed by the curve for urea in Fig. 3, where the penetration of urea from blood into the perfused fluid is plotted against time after the concentration of urea in the blood has been established at a steady level. If exchanges between blood and nervous tissue were rapid, the concentration in the perfused fluid should achieve a value such that the ratio (concn. in fluid):(concn. in plasma) was 0·29 times the value that is achieved when the brain has been allowed

to equilibrate with the blood for 24 hr before beginning the perfusion. In fact the ratio is only about 0·14 at 2 hr and still rising fairly rapidly. A similar situation exists for ^{24}Na (Davson & Pollay, 1963). Thus to enter perfusion fluid much of the ^{14}C-urea must first enter brain and this is a slow process (Bradbury & Coxon, 1962; Reed & Woodbury, 1962; Kleeman *et al.* 1962), hence the delay in equilibration. It is doubtful whether the ratio 0·29, in fact, represents the true steady state, as urea is probably still being leached out of brain at 2 hr (Fig. 2). The kinetics of equilibration for ^{14}C-urea in Fig. 3 are obviously complex, with the rate of perfusion influencing the rate of equilibration of ^{14}C-urea in brain, and no attempts have been made to derive kinetic equations. It is to be noted that even if a steady state for the equilibration of ^{14}C-urea and total urea has not been reached in Fig. 4, equation (8) is still valid, as the diffusion components of ^{14}C-urea out and total urea in will be both affected equally.

Thus the transport of urea between blood, brain and cerebrospinal fluid may be completely described in terms of diffusion and entrainment with streams of fluid. This concept is at variance with reports that in the steady state the concentration of urea in grey matter may be greater than that in blood and that active transport of urea into neurones may occur (Bradbury & Coxon, 1962; Kleeman *et al.* 1962). However, later studies in which ^{14}C-urea was injected intravenously into rabbits, and their brains were analysed by a method specific to this compound 48 hr later, gave a (brain water):(plasma water) ratio for urea in grey matter of 0·97, a finding that makes the occurrence of active transport of urea in brain unlikely (Bradbury, 1962).

Creatinine. The experiments in which creatinine was perfused at the low and the high concentrations again provide no evidence of a saturable system being involved in its transport out of the fluid, the mean K_{out} being 0·0049 at low concentration and 0·0059 at high concentration respectively. The transfer constants are over half those for urea. Heisey *et al.* (1962) found the constant for creatinine to be rather less than a third of the constant for urea in the unanaesthetized goat. Since solutes must diffuse across brain tissue to leave the fluid, the difference may well be due to anatomical differences between the two species.

The concentration of creatinine in the effluent, when the blood concentration is raised, is again dependent on whether time is allowed for creatinine to pass into brain before the perfusion is begun. Thus when intravenous infusion and perfusion are started together (Fig. 3), the concentration of creatinine in the effluent reaches 0·044 of that in blood in 2 hr. When the blood level has been maintained constant for 24 hr before perfusion (Fig. 2), the concentration in the effluent at 2 hr is 0·084 of that in the blood. A similar mechanism to that operative in the case of urea

may be postulated. If entry of creatinine into brain is very slow, the different curves will be explained.

When a raised blood level of creatinine is maintained for 24 hr and no perfusion is made, the average concentrations of creatinine in brain water and cerebrospinal fluid are 0·33 and 0·17 of that in plasma. The most likely explanation for this is that cerebrospinal fluid is acting as a sink for creatinine. If the newly formed cerebrospinal fluid contains little creatinine and diffusion between brain and fluid is much freer than between blood and brain, much of the creatinine that enters brain from blood will quickly drain into cerebrospinal fluid and be carried away with the flowing fluid. Eventually an equilibrium will be reached with the relative concentrations being in the order: blood > brain > cerebrospinal fluid. This system may well have functional significance. If no drainage system was available, any polar molecules of moderate weight produced by metabolism will tend to accumulate in brain, as such substances pass the blood–brain barrier with difficulty (Brodie, Kurz & Schanker, 1960). This system allows them to drain into cerebrospinal fluid and be carried away.

The volume of distribution of creatinine in the body is close to the volume of the total body water (Schloerb, 1960), so brain and cerebrospinal fluid must be exceptional in containing creatinine at much lower concentrations than those in blood plasma. This anomaly is, as has been discussed, a reflexion of the unusual barrier and drainage system of brain and cerebrospinal fluid.

Monosaccharides. The system involved in the exit of glucose and xylose from the perfusion fluid is saturable. The K_{out} for ^{14}C-glucose in tracer quantities is 0·0191, but when the concentration of glucose in the perfusion fluid is raised to 34 mM the constant is reduced to 0·0101. Changing from the low to the high concentration of xylose also results in a significant depression of its transfer constant. Further, there is competition between the two sugars; 34 mM xylose will reduce the K_{out} for tracer ^{14}C-glucose to 0·0127. The K_{out} for ^{14}C-fructose or total fructose at the low concentration is much less than that for the other two sugars, but it is further reduced by glucose at the high concentration. Glucose and fructose are metabolized rapidly by minced brain *in vitro*, but the oxidation of xylose is not detectable (Page, 1937). Thus, although the self-saturation and competitive inhibition of ^{14}C-glucose and ^{14}C-fructose exit from the fluid could be due to interference with an enzyme system, concerned in metabolism rather than transport of the sugars, the self-inhibition of xylose exit must be due to saturation of a transport system. It is likely that all the sugars studied are transported by a common carrier system and that transport against a concentration gradient does not occur. That energy is not required is indicated by the following: first, dinitrophenol (0·05 mM) in

the perfusion fluid causes no decrease in the rate of removal of ^{14}C-glucose from the fluid; the small increase observed, if not a chance finding, could be due to increased oxidation of glucose by brain in the presence of DNP. Secondly, the K_{in} for the entry of glucose into the perfusion fluid, when a glucose-free or tracer-containing fluid is perfused, is of similar size to the K_{out} for ^{14}C-glucose. Xylose, too, appears to equilibrate fairly rapidly in between the perfusion fluid and the blood, when the concentration in the latter is raised and maintained constant by intravenous infusion (Fig. 3). In fact, xylose reaches a concentration that is 0·33 of that in blood in 2 hr, whereas the concentration of glucose in the fluid falls to 0·34 in 2 hr. The entry of xylose (Fig. 3) is so much faster than that of the less polar ^{14}C-urea (Fig. 3) that a specialized system must be involved in its entry as well as its exit.

That no entry of fructose into the perfusion fluid could be demonstrated when the blood concentration of this sugar is raised to 2·0 mM, fits with the observation of Klein, Hurwitz & Olsen (1946) that the blood level of this sugar had to be raised to levels of more than 10 mM for periods of more than 30 min before it was detectable in brain. Slow transport and rapid utilization must combine to keep brain and cerebrospinal fluid levels very low.

It would require many experiments, performed with different concentrations of sugar in blood and perfusion fluid, together with analyses for the sugars in brain tissue, completely to characterize the carrier system. However, the exit experiments do indicate that the affinity of the carrier for sugars varies in the order: glucose > xylose > fructose, and it could be that the carrier is the same as that involved in the transport of sugars across the red blood cell membrane (LeFevre & Davies, 1951; Widdas, 1954; Wilbrandt, 1956). Our experiments provide no evidence as to whether this mechanism is a general feature of the cells of the choroid plexuses, ependyma and nervous tissue or whether it is localized to certain sites. The results obtained in the exit experiments could be explained by the presence of the carrier at the blood–brain barrier rather than between fluid and brain. That transport of sugars across this barrier is carrier mediated has been proved by Crone (1961).

One further point requires comment. The curves for glucose and urea in Fig. 2 cross at about 50 min. The concentration of glucose in cerebrospinal fluid relative to its concentration in blood is less than the concentration of urea. After 50 min perfusion the concentration of glucose in the effluent is greater than that of urea. Transfer of glucose between brain and fluid is more rapid than transfer of urea (Table 1). Hence in Fig. 2 one might expect the curve for glucose to be well above that for urea at all times. That they are more together and in fact cross is readily explicable,

if one supposes that the glucose concentration in brain is much less than that in blood. This has been proved to be so (Kerr & Ghantus, 1936; Klein *et al.* 1946). The former authors found a brain:plasma ratio of 0·41 for the rabbit. This must be due to entry of glucose from blood failing to balance the rapid utilization of glucose by brain, unless a considerable concentration gradient is present.

Thus the low concentration of urea in cerebrospinal fluid is due to the circumstance that the primarily secreted fluid from the choroid plexuses has a low concentration of urea, say, 0·6 times that of the plasma. This concentration increases slowly, as the fluid passes down the neural axis, by diffusion from the nervous tissue which contains a similar concentration of urea to that in blood. The normal low glucose in cerebrospinal fluid may be primarily due to the low concentration in brain impressing itself by easier exchange on the cerebrospinal fluid. This is supported by the fact that, in contrast to urea, there is no difference in the glucose content of human ventricular and lumbar fluids (Marks, 1960), nor are there marked differences in its content in successive samples of fluid withdrawn from the cisterna magna of the cat and rabbit (Davson, Kleeman & Levin, 1961).

SUMMARY

1. Artificial cerebrospinal fluid was perfused between the lateral cerebral ventricle and the cisterna magna of the anaesthetized rabbit. This system was used to study the exchanges of urea, creatinine, D-glucose, D-xylose and D-fructose between blood, brain and cerebrospinal fluid.

2. Urea appears to enter the fluid unidirectionally with the newly formed cerebrospinal fluid at a concentration of about 60% of that in plasma. Diffusional exchanges then occur between blood and cerebrospinal fluid, largely across nervous tissue.

3. Little creatinine enters the fluid with the newly formed cerebrospinal fluid and subsequent diffusional exchanges between blood, brain and fluid occur at a slower rate than those of urea.

4. The major impediment to the diffusion of these substances must lie between blood and brain, rather than between cerebrospinal fluid and brain.

5. Transport of the monosaccharides, D-glucose and D-xylose, and probably D-fructose, in and out of cerebrospinal fluid is carrier-mediated. No evidence was found for transport against a concentration gradient.

6. The affinity of the carrier for the sugars studied varies in the order: glucose > xylose > fructose.

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