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UPTAKE OF [^{14}C]UREA BY THE *IN VIVO* CHOROID PLEXUS–CEREBROSPINAL FLUID–BRAIN SYSTEM: IDENTIFICATION OF SITES OF MOLECULAR SIEVING

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SUMMARY

1. The time course of the uptake of [^{14}C]urea by the lateral ventricular choroid plexus of the adult rat *in vivo* was analysed to delineate further the permeability characteristics of the epithelial membrane of this secretory tissue.

2. Eight hours after I.P. injection, [^{14}C]urea attained a steady-state distribution in 78% of the tissue water of lateral ventricular choroid plexus; similarly, approximately 8 hr was required for radiourea to reach a steady-state concentration in both the cerebral cortex and cerebrospinal fluid (c.s.f.).

3. Results obtained for compartment analysis were used to calculate the concentration of [^{14}C]urea in the epithelium of the lateral ventricular plexus during the approach to and at steady-state distribution. Even after 1 hr of distribution, the [^{14}C]urea concentration in choroid cell water was less than 15% of that in plasma water.

4. Although the concentration of radiourea in choroid cell water continually increased after 3 hr, it remained in equilibrium with the concentration of [^{14}C]urea in c.s.f. water. At the steady state (i.e., 8 hr), the distribution of [^{14}C]urea between the water of plasma and that of the choroidal epithelium was considerably away from equilibrium (i.e., by 25–30%).

5. An analysis of the concentration gradients for [^{14}C]urea across both the apical (c.s.f.-facing) and basolateral (plasma-facing) membranes of the epithelium of the lateral ventricular plexus suggests that the movement of urea is hindered to a greater extent by the basolateral membrane than by the apical membrane.

6. Only a single half-time component (1·3 hr) can be resolved from analysis of the curve describing the time course of uptake of radiourea by the choroid epithelial cell compartment.

7. The concentration gradient data suggest that urea penetrates from blood to c.s.f. via the choroid plexus by a transcellular pathway; however, it is not possible to rule out a paracellular pathway for urea movement.

8. At the steady state, radiourea distributes into 88% of the water of cerebral cortex. This observation, together with the finding of a steady-state concentration gradient for [^{14}C]urea from cortical tissue to c.s.f., constitutes evidence that urea movement is hindered at the blood-brain barrier as well as at the blood–c.s.f. barrier.

INTRODUCTION

The relatively slow movement of urea from blood into various compartments of the central nervous system (C.N.S.) is in marked contrast to the rapidity with which this hydrophilic molecule penetrates non-neural tissues, e.g. skeletal muscle (Bradbury & Coxon, 1962). The observation that several hours are required for [^{14}C]urea tracer molecules to achieve a steady-state distribution between plasma, brain and cerebrospinal fluid (c.s.f.) has been attributed to the structural nature of membranes which interface the plasma with the brain and with the c.s.f. (Cserr, Fenstermacher & Rall, 1970). Electron micrographs reveal the presence of tight junctions interposed between the epithelial cells of the choroid plexus, i.e., the blood-c.s.f. barrier, and between the endothelial cells of the cerebral capillaries, i.e., the blood–brain barrier (Brightman & Reese, 1969). The tight junctions in the cerebral capillaries probably impede the diffusion of even relatively small molecules like urea (Cserr *et al.* 1970; Fenstermacher, Patlak & Blasberg, 1974). In contrast, transport studies by Welch & Sadler (1966) and by Wright & Prather (1970) who utilized *in situ* and *in vitro* preparations, respectively, have furnished evidence that the choroid plexus is relatively permeable to urea; thus, the penetration of small polar solutes into the C.N.S. is a problem which deserves further analysis.

There have been no investigations to analyse the time course of uptake of a non-electrolyte such as urea by the *in vivo* choroid plexus. The focal point of the present study has been to delineate further the permeability characteristics of the epithelium of the choroid plexus from the analysis of the kinetics of *in vivo* uptake of [^{14}C]urea by this secretory tissue. By analysing the kinetics of distribution of tracer urea between blood, choroid plexus, c.s.f. and cerebral cortex, we have attempted to define the functional interrelationships between the blood–c.s.f. and blood–brain barriers with respect to the movement of urea. To facilitate the interpretation of radiourea distribution in the C.N.S., the uptake of tritiated water has also been analysed.

METHODS

Animals and experimental protocol. Sprague–Dawley male rats (300–425 g) were used in all aspects of the study. Each animal was anaesthetized by the insertion of its nose into a cone containing an ether-saturated gauze pad. Before the I.P. administration of a radioisotope, each rat was bilaterally nephrectomized by ligation of the renal pedicles. A total of fifty-six rats was divided into groups of forty-four and twelve animals which were injected with [^{14}C]urea (45 μc) and [^{3}H] water (5 μc), respectively; both radioisotopes, which were obtained from the New England Nuclear Corp., were dissolved in 0·9 % (w/v) NaCl solution. Rats injected with [^{14}C]urea were sacrificed at 0·5, 1, 2, 3, 5, 8, 11·5 and 16 hr after administration of isotope; those that received tritiated H_2O were killed at 3, 6, 9 and 15 min after injection.

To analyse the change in blood content of urea and electrolytes subsequent to nephrectomy, eight additional animals were bilaterally nephrectomized and sacrificed at either 0·5 or 8 hr post nephrectomy. Because of diurnal variations in the plasma concentration of urea (Gabriel, 1975), it was considered desirable to sacrifice all of these nephrectomized animals as close together in time as feasible, i.e. within an hour.

Sampling of tissues and fluids. At the termination of an experiment, blood was withdrawn from the abdominal aorta into a heparinized syringe before severing this artery; immediately thereafter, a 0·1 ml. sample of c.s.f. was aspirated from the cisterna magna into a 0·25 ml. syringe. After removing the brain, a surgical blade was used to shave a 1 mm layer of cerebral cortex from frontal and parietal regions. Subsequently, the choroid plexus in each lateral ventricle was

removed with a pair of fine forceps ('lateral ventricular choroid plexus' is hereafter referred to as 'choroid plexus'). The microtechniques involved in the sampling and weighing of small pieces of choroid plexus have been described elsewhere (Johanson, Reed & Woodbury, 1976). For comparative purposes, skeletal muscle was sampled.

Sample processing and analytical techniques. Samples of fluids and tissues were digested overnight in 1 M-piperidine at 60 °C. One-half ml. of each digested sample was added to 15 ml. scintillation cocktail consisting of diphenyloxazole, toluene and ethoxyethanol. A Nuclear-Chicago Isocap-300 counter, on line with a PDS/3 data processor programmed to determine radioisotope counting efficiency by the channels ratio method, was employed to determine the radioactive disintegrations in each sample. Plasma samples were analysed for Na and K with an Instrumentation Laboratory Flame Photometer. The pH and p_{CO_2} of anaerobically-collected blood samples were determined by use of an Instrumentation Laboratories blood-gas apparatus. An Abbot Biochemical Analyzer was utilized for the measurement of levels of urea and glucose in plasma. Equilibrium dialysis studies were performed to determine the degree of binding of [^{14}C]urea to components of plasma and tissue. Samples of plasma and tissue homogenates containing [^{14}C] activity were dialysed against a synthetic c.s.f. containing neither protein nor radioactivity. All samples were dialysed for 20 hr at constant temperature (37 °C).

Calculations and analysis of data. The extent of uptake (or distribution) of a given radioisotope by a particular tissue or c.s.f. is expressed as a space calculated by

$$\frac{\text{dpm/mg tissue } H_2O \text{ or c.s.f. } H_2O}{\text{dpm/mg plasma } H_2O}.$$

The relatively slow uptake of [^{14}C]urea by various compartments in the C.N.S. makes it feasible to plot and to analyse the [^{14}C]urea spaces as a function of time after administration of tracer. Because of the rapidity of uptake of [^{3}H]H_2O, the time course of distribution of this tracer in the present experiments does not readily lend itself to kinetic analysis; thus, the [^{3}H]H_2O spaces are presented as tabular data.

The [^{14}C]urea spaces, together with results obtained for compartment analysis for choroid plexus H_2O content, extracellular fluid volume ([^{3}H] inulin space) and residual erythrocyte volume (^{51}Cr-tagged-erythrocytes) obtained previously (Johanson & Woodbury, 1978), have been utilized to calculate the concentration of radiourea in the choroidal epithelium; see references by Johanson *et al.* (1974, 1976, 1978) for a discussion of the assumptions and equations pertaining to the calculation.

RESULTS

Levels of [^{14}C]urea and other substances in the plasma of nephrectomized rats

The time course of activity of [^{14}C]urea in the plasma of nephrectomized rats injected with tracer for periods ranging from 0·5 to 8 hr was analysed by linear regression; the slope of the regression is not statistically significant ($P = 0{\cdot}68$). Thus, nephrectomy is effective in stabilizing the level of [^{14}C]urea in plasma for the period of time required for [^{14}C]urea to achieve a steady-state distribution in the C.N.S. Accordingly, the calculated [^{14}C]urea spaces are based upon a level of [^{14}C]urea in plasma which is not fluctuating significantly.

Subsequent to nephrectomy, the concentrations of some solutes in the plasma are altered. Eight hr after ligation of both renal pedicles, the concentration of K in plasma is elevated by approximately 2 m-equiv/l. ($P < 0{\cdot}001$). However, there are no statistically significant changes (0·5 *vs.* 8 hr) in plasma Na or in arterial pH and p_{CO_2}. Plasma urea rises to 37 mg/dl. ($P < 0{\cdot}05$). The concentration of glucose in plasma falls by about 30% during this period of nephrectomy ($P < 0{\cdot}05$).

[^{14}C]urea uptake by various compartments in the central nervous system

Following the I.P. injection of [^{14}C]urea, approximately 8 hr elapses before this tracer reaches a steady-state distribution in the choroid plexus. The curves in Fig. 1

show that the rate of uptake of radiourea by the choroid plexus is relatively slow compared with that by skeletal muscle. With respect to extent of uptake, [^{14}C]urea distributes into only 0·78 of the tissue H_2O of choroid plexus; for comparison, [^{14}C]urea distributes into all of the H_2O of skeletal muscle by 1 hr post injection. The

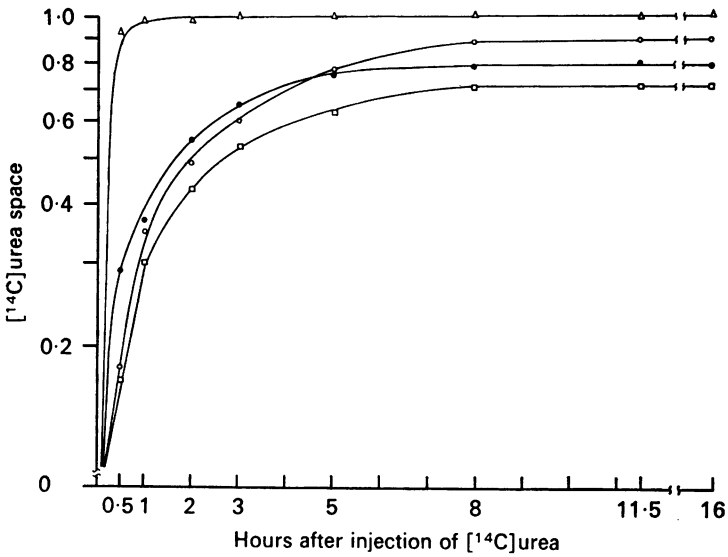

Fig. 1. Time course of uptake of [^{14}C]urea by tissues and c.s.f. in the adult rat. The [^{14}C]urea space plotted on ordinate is defined in text. Each plot on graph for skeletal muscle (△), cerebral cortex (○) and choroid plexus (●) is a mean value for five or six rats; for c.s.f. (□), each plotted mean value represents four or five animals. Standard errors are generally < 5% of the respective means.

pattern of uptake of [^{14}C]urea by cerebral cortex is similar to that of the choroid plexus. Slightly more than 8 hr is required for this tracer to attain steady-state distribution in cortical tissue. At the steady state, [^{14}C]urea is distributed into 0·88 of the H_2O of the cerebral cortex. The curve describing the uptake of [^{14}C]urea by the c.s.f. runs more-or-less parallel to corresponding curves for plexus and cortex. Nearly 8 hr of distribution is necessary for tracer urea to build up to its steady-state concentration in c.s.f. The magnitude and direction of the displacement of the asymptotes suggest that at the steady state there is a sink effect for the distribution of [^{14}C]urea in the direction from plasma to choroid plexus to c.s.f. as well as from plasma to cortex to c.s.f.

Compartmental analysis of [^{14}C]urea distribution

The relative concentrations of [^{14}C]urea in the epithelial cell compartment of the lateral ventricular choroid plexus, together with tracer concentrations in those fluids which bathe either side of the epithelial membrane, are presented in Table 1. The time-course analysis of changes in the concentration of [^{14}C]urea in choroid cell H_2O and c.s.f. H_2O (relative to plasma H_2O) provides indirect evidence about the nature of urea movement among the compartments. With respect to Table 1, four points are considered important: (i) even after 1 hr of distribution, the [^{14}C]urea concentration in choroid cell H_2O is substantially lower than in plasma and c.s.f. H_2O;

(ii) approximately 2–3 hr is necessary for [^{14}C]urea to equilibrate between the H_2O of the choroidal epithelium and the H_2O of the c.s.f.; (iii) although the concentration of radiourea in choroid cell H_2O continually increases up to 8 hr, after 3 hr it remains in equilibrium with the concentration in c.s.f. H_2O; (iv) at the steady state (8 hr and after), the distribution of [^{14}C]urea between plasma H_2O and choroid cell H_2O is away from equilibrium.

TABLE 1. Relative concentrations of [^{14}C]urea in various compartments*

	Hours after I.P. injection of [^{14}C]urea							
	0·5	1	2	3	5	8	11·5	16
Plasma H_2O	100	100	100	100	100	100	100	100
Choroid epithelial cell H_2O	2 ± 1	14 ± 1	38 ± 2	52 ± 3	65 ± 1	72 ± 1	72 ± 3	71 ± 4
Cerebrospinal fluid H_2O	17 ± 1	30 ± 1	43 ± 2	52 ± 3	62 ± 1	71 ± 1	71 ± 1	72 ± 1

* Data listed in table are relative concentrations of [^{14}C]urea in various compartments based upon a plasma concentration of 100; for example, at 1 hr the concentration of [^{14}C]urea in c.s.f. H_2O is 30% that in plasma H_2O. Each tabular entry for choroid cell H_2O (or for c.s.f. H_2O) represents a mean concentration of [^{14}C]urea in that compartment which has been calculated from individual values of radio-urea spaces (Fig. 1) and pooled values of compartmentation data (Johanson & Woodbury, 1978). Limits are standard errors.

TABLE 2. Half-time data for curves describing the uptake of [^{14}C]urea by various compartments in the central nervous system

Compartment	Half-times			
Choroid plexus* (whole tissue)	0·15 hr	(0·09);‡	1·2 hr	(0·91)
Choroid plexus† (epithelium)	—	— ;	1·3 hr	(~ 1)
Cerebrospinal* fluid	0·3 hr	(0·21);	2·0 hr	(0·79)
Cerebral cortex* (whole tissue)	0·4 hr	(0·16);	2·1 hr	(0·84)

* Curve parameters obtained from analysis of curves in Fig. 1.

† Curve parameters obtained from analysis of the curve plotted from data in Table 1 for [^{14}C]urea concentration in choroid epithelial cell H_2O.

‡ Data in parentheses are volumes of distribution (V_d) which correspond to the respective half-times; each V_d value, i.e., a y-intercept obtained from extrapolation of a plot of data obtained by curve subtraction, is expressed as a fraction of the asymptote (steady-state plateau) of an uptake curve.

Analysis of uptake curves

Table 2 contains data for half-times and volumes of distribution (V_d) obtained from analysis of curves describing the time course of uptake of [^{14}C]urea. With respect to choroid plexus, c.s.f. and cerebral cortex, two half-time components were resolvable from each of the respective curves in Fig. 1. For c.s.f. as well as for cerebral cortex, uptake half-times of approximately 0·35 and 2·0 hr were obtained from the curve analysis; for choroid plexus (whole tissue), half-times of 0·15 and 1·2 hr were found. In general, the V_d associated with the shorter half-times were approximately

10–20% of the V_d associated with the longer half-times. The curve describing the uptake of radiourea by the epithelial cell compartment of the choroid plexus resolved into a single component, a half-time of 1·3 hr.

Binding of tracer urea

Consideration of binding of urea is pertinent to the interpretation of the data in Table 1. To evaluate the degree of binding of [^{14}C]urea to plasma and tissue components, the technique of equilibrium dialysis was employed. Neither *in vitro* nor *in vivo* studies of plasma or tissue revealed evidence for significant binding of ^{14}C activity.

TABLE 3. Uptake of tritiated water by various tissues and cerebrospinal fluid in the adult rat*

	Minutes after I.P. injection of [^{3}H]H_2O				
	3	6	9	15	*P* value†, 9 *vs.* 15 min
[^{3}H]H_2O spaces:					
Cerebrospinal fluid	0·65	0·89	0·95	0·97	> 0·05
Choroid plexus‡	0·82	0·95	1·02	0·99	> 0·05
Cerebral cortex	0·87	0·96	0·97	0·99	> 0·05
Skeletal muscle	0·59	0·93	1·00	1·00	> 0·05

* Extent of uptake expressed as space as calculated by the formula in Methods section. Each value represents a mean for three to five rats. Standard errors are generally within the range of 2–6% of the respective means.

† Statistical significance determined by multiple range test.

‡ Because of the problem of evaporation of water from the small-sized samples of choroid plexus (see Methods), [^{3}H]H_2O spaces were not measured in this tissue. The spaces for choroid plexus are those determined from the distribution of [^{14}C]antipyrine.

Transport studies with tritiated water

The space data in Table 3 indicate that [^{3}H]H_2O penetrates rapidly even into those compartments in the C.N.S. that are relatively impermeable to urea. Whereas several hours is required for [^{14}C]urea to attain a steady-state distribution in the C.N.S., only several minutes is necessary for [^{3}H]H_2O to attain the same. Presumably because of blood flow-limited distribution, tritiated-H_2O permeates skeletal muscle less rapidly than it diffuses into the C.N.S.

DISCUSSION

Uptake of [^{14}C]urea by the in vivo choroid plexus

Although blood flow (per unit weight of tissue) to the choroid plexus is substantially greater than that to skeletal muscle (Welch, 1963; Goldstein, Aronow & Kalman, 1969), the uptake of radiourea by the choroid plexus is relatively slow compared with that by muscle. Evidence that the slow uptake of [^{14}C]urea by the plexus is not due to sluggish arterial perfusion is provided by the observation that radioantipyrine penetrates rapidly into the total H_2O of this tissue (Table 3). Since the capillaries of the plexus are fenestrated (Maxwell & Pease, 1956), it seems safe to rule out the choroidal capillary wall as a rate-limiting barrier to the diffusion of urea. The half-time of 0·15 hr probably represents the rapid distribution of urea into the e.c.f. of the plexus; the V_d of about 0·1 which corresponds to this half-time is a reasonable

approximation of the volume of e.c.f. of the lateral ventricular plexus (Johanson, Reed & Woodbury, 1976). Thus, the slow build-up in [^{14}C]urea concentration in the plexus is presumably related neither to blood flow-limited distribution nor to a permeability barrier in the choroidal capillaries.

Concentration of radiourea in choroid plexus and c.s.f.

The uptake curves indicate that there is always a concentration gradient for [^{14}C]urea from the choroid plexus (whole tissue) to c.s.f. However, the [^{14}C]urea space in the choroid plexus (Fig. 1) is uncorrected for [^{14}C] activity in residual erythocytes; when this sizeable correction (Johanson *et al.* 1978) is made in the calculation of cellular concentrations (Table 1), it becomes apparent what the gradient of [^{14}C]urea is across the choroidal epithelium. One interpretation of the concentration gradients (derivable from data in Table 1) is that the c.s.f. is a significant *source* of [^{14}C]urea for the choroidal epithelium. However, it is improbable that during the approach to steady-state distribution there is a net flux of tracer urea from c.s.f. to choroidal epithelium because of the bulk flow of secreted c.s.f. which would oppose such a movement of urea. The single half-time component that can be resolved from the [^{14}C]urea curve for the choroidal epithelium (Table 2) suggests that under the experimental conditions there is only one quantitatively significant route of penetration of tracer into the epithelium. Because of solvent drag associated with c.s.f. secretion, it seems not unreasonable to conclude that such a route for net flux (during the attainment of steady-state distribution) of tracer urea is from plasma into the choroid cell via the basolateral membrane. A flow of urea less rapid than that of H_2O (Bradbury & Davson, 1964) from blood to epithelial cells, i.e., a sieving effect, is presumably why [^{14}C]urea in the choroid cell is kept lower than in the plasma.

Permeability of the choroid plexus to urea

The data in Table 1 suggest that the apical membrane of the choroid cell is an interface that allows the relatively free distribution of urea between the epithelium of the plexus and the c.s.f.; on the other hand, the basolateral membrane of the choroidal epithelium is probably not as readily permeable to urea. One-half hour after I.P. injection, there is only negligible [^{14}C]urea activity in the choroid cell; at this time, [^{14}C]urea is present in muscle at 90 % of its steady-state concentration. Because of the complicating factor of a rapid turnover of water in the choroid plexus, it is not possible by comparing concentration data to conclude that the choroid cell is less permeable to urea than is the muscle cell. However, these observations, together with the finding that there is never a concentration gradient for [^{14}C]urea from choroid cell to c.s.f., imply that the basolateral membrane substantially hinders the permeation of the urea molecule from plasma to the epithelial cell compartment. If the basolateral permeability to urea were substantially greater than that of the apical membrane, one would expect that, following I.P. injection, [^{14}C]urea would distribute in most of the H_2O in the choroid plexus. However, the concentration of [^{14}C]urea in choroid cell H_2O is never greater than 72 % of its concentration in plasma H_2O (Table 1).

Considerations important to the interpretation of the data

The foregoing conclusions are dependent upon the following assumptions: (1) that the [^{14}C]activity calculated for the choroid cell is associated with urea (unbound), (2) that there is not carrier-mediated transport of urea in the rat choroid plexus and (3) that the concentration of urea in the cisternal fluid is similar to that in the ventricular fluid. The findings of several investigations of potential metabolite formation from urea (Gilboe, Javid & Frechette, 1960; Schoolar, Barlow & Roth, 1960; Reed & Woodbury, 1962; Rasmussen, 1971), together with the results obtained from our binding study, allow us to be confident of the first assumption. The second assumption is upheld by the observation of Bradbury & Davson (1964) that carrier urea up to concentrations of 34 mM does not affect the clearance of [^{14}C]urea from mammalian c.s.f.; also, in man there is no active transport of urea during c.s.f. formation (Bradbury, Stubs, Hughes & Parker, 1963); additional evidence (Davson, Kleeman & Levin, 1962; Pollay & Kaplan, 1970) indicates that the movement of urea among the compartments in the C.N.S. occurs by diffusion. Thus, the elevated concentration of endogenous urea in nephrectomized rats should not affect the distribution of tracer urea in the brain. The third assumption, concerning the chemical nature of sampled c.s.f., is supported by compositional data reported by Kleeman *et al.* (1962).

Moreover, it is known that high concentrations of urea affect neither the activity of carbonic anhydrase in the choroid plexus (Fisher, Copenhaver & Maline, 1959) nor the rate of formation of c.s.f. (Kleeman *et al.* 1962). The alteration in the plasma levels of electrolytes is probably not sufficiently marked to modify the permeability of cellular membranes.

Penetration of [^{14}C]urea into c.s.f. and brain

The present study has furnished evidence that urea can gain access to the c.s.f. via a transcellular pathway in the choroid plexus. Similarly, Wright & Pietras (1974) concluded that there is transcellular penetration of urea through the frog choroid plexus. Although it is not possible to distinguish between transcellular and paracellular routes (Smulders & Wright, 1971) on the basis of the half-time data, the relatively short half-time of 0·3 hr for the rate of appearance of tracer urea into the c.s.f. probably represents primarily the movement of urea across the choroid plexuses. The longer half-time of 2·0 hr for c.s.f. is similar to that for cerebral cortex; thus, it seems likely that the c.s.f. is following the brain, the c.s.f. being dependent upon entry of urea across the blood-brain barrier.

The rapidity of penetration of [^{3}H]H_2O into the cerebral cortex indicates that blood flow to this region is not a limiting factor in the distribution of tracers. Nevertheless, the uptake of [^{14}C]urea by the cortex is relatively slow compared to muscle. The V_d of urea into approximately 16 % of the H_2O of the cerebral cortex, together with the associated half-time of 0·4 hr, probably represents primarily the early spreading of this tracer into the extracellular fluid. At the steady state, radiourea is distributed in 88 % of the H_2O of cerebral cortex; thus, our findings for the rat are similar to those of Bradbury & Coxon (1962) for the cat and rabbit, i.e., tracer urea does not distribute at equilibrium between the H_2O of plasma and that of cerebral

cortex. However, the brain: c.s.f. spaces for [^{14}C]urea, which can be calculated from data in Fig. 1, suggest that there is a relatively free exchange of urea between cortex and c.s.f. Thus, the collective data suggest that there is a hindrance to the movement of urea through the cerebral capillary wall. Moreover, data obtained from other preparations (Coxon, 1968; Cserr *et al.* 1970; Fenstermacher *et al.* 1974) indicate substantial differences among various species with respect to the penetration of urea at the blood–brain and blood–c.s.f. barriers (Cserr, 1974). Further work is required to ascertain the significance of species differences in the rate and extent which small polar solutes gain access to the C.N.S.

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