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Penetration of ^{14}C-Inulin and ^{14}C-Sucrose into Brain, Cerebrospinal Fluid, and Skeletal Muscle of Developing Rats*

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Summary. 1. We studied the decrease in brain uptake of two substances, inulin and sucrose, during maturation of the brain. Concentration ratios of CSF/plasma and brain/plasma for ^{14}C-labeled inulin and sucrose were calculated and time-uptake curves plotted for prenatal and postnatal rats. Comparisons of these ratios among the various ages showed a progressive decrease in the inulin ratios of both compartments with increasing age. Sucrose "spaces" were always larger than inulin "spaces," suggesting possible sucrose penetration into cells. The brain/CSF concentration ratio for inulin, however, appears to reflect brain extracellular space in young rats, but not in older animals. Electrolyte concentration ratios of CSF and plasma suggested progressive maturation of transport mechanisms at the choroid plexus and increase of CSF flow. The degree of concentration of inulin in the developing brain and the CSF of the young rat probably reflects cellular maturation of both barrier and secretory mechanisms.

2. We also measured the inulin and sucrose space of skeletal muscle in rats of different age. In contrast to brain, the spaces measured by the two substances were the same and decreased with maturation of the animals.

Key Words: Blood-brain barrier — Cerebrospinal fluid — Skeletal muscle — Electrolytes — Maturation — Extracellular Space

Introduction

Substances such as inulin and sucrose that distribute extracellularly in the body should prove useful in measuring the extracellular space of the brain. However, even when maintained at steady levels in the plasma for long periods of time, these substances yield values that underestimate the functional brain extracellular space, at least in adult animals. The low value is due to a "sink effect" in the cerebrospinal fluid (CSF) which is greater than the rates at which the substances penetrate into the brain (DAVSON 1963; REED *et al.* 1965). Thus, in adult animals, slow penetration across the brain capillaries and more rapid drainage into the CSF combine to prevent certain plasma solutes from reaching equilibrium in brain interstitial fluid.

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The brain uptake of various plasma solutes diminishes with increasing age. Additional mechanisms to those operative in the adult may reduce brain uptake in the immature or young animal. In the case of cerebral metabolites, for example, changing uptake may reflect the metabolic state of the growing brain (Dobbing 1961). For a substance such as inulin, decreasing size of the extracellular space of the brain would result in diminishing concentration in the brain. In the present study an attempt was made to elucidate various factors that contribute to reduced uptake of inulin and sucrose by the maturing brain. Although a previous study dealt with brain uptake of labeled inulin and chloride in developing rats (Vernadakis and Woodbury 1965), it did not include simultaneous measurement of uptake by the CSF. Spinal fluid concentrations have been measured in the present study. In addition, changes with age in the composition of CSF electrolytes were determined to evaluate the possible effects of CSF secretion on uptake of inulin and sucrose by the brain and spinal fluid. Inulin and sucrose were used because these water-soluble, non-metabolizable carbohydrates are presumably confined to the extracellular space of tissues (Woodbury 1965a).

Methods

General. Prenatal, newborn and postnatal Sprague-Dawley rats were used in the experiments. Inulin or sucrose labeled with ^{14}C was administered intraperitoneally to rats of various ages or into mothers in the case of the newborn and fetal animals. The injected animals were bilaterally nephrectomized in order to maintain plasma concentrations at steady levels during the experiments. With one exception all injections were made into rats nephrectomized 24 hours before sacrifice. At selected times after i.p. injection, samples of CSF, blood and cerebral cortex were taken simultaneously from each animal for determination of radioactivity and for electrolyte analyses. Operative procedures and removal of samples were performed under brief ether anesthesia. Cerebrospinal fluid was obtained by cisternal puncture with glass micropipettes handmade from capillary tubing. The volumes of CSF obtained were 5—10 μl in prenatal, 20—50 μl in 8-day-old and up to 50 μl in older animals. In the case of the prenatal animals an incision was made in the uterine wall and fetal membranes to expose the fetus. With the animal still attached by its placenta the cisternal puncture was made to obtain the CSF sample. Blood was drawn either from the abdominal vessels (usually aorta, but occasionally the vena cava in very young rats) or from the heart into heparinized micropipettes that were centrifuged to obtain the plasma. The cerebral hemipheres were removed and blotted to remove blood. In most cases, samples of CSF, blood and cerebral cortex were taken at 2, 4, 8, 16 and 24 hours after the intraperitoneal injection of 5—20 μc of ^{14}C-inulin or ^{14}C-sucrose. A few groups of animals received the injections 30 min before sacrifice; one group of 3-day old rats were nephrectomized and received the tracer 36 hours prior to sacrifice. A separate study of the effects of nephrectomy on CSF and brain uptake by 9-day old rats was performed; in this case, injections of inulin were given at selected times after nephrectomy (see Results).

Determination of Radioactivity. The tissues were prepared for counting as follows. The micropipette used for the CSF sample was weighed on a single pan balance sensitive to 0.02 mg before and after removal of the sample to determine its weight by difference; the sample was expelled into planchets containing 1 ml of distilled water and dried for counting. The plasma obtained after centrifugation of the micropipettes was drawn into calibrated micropipettes and transferred to planchets for drying and counting. The cerebral hemispheres were placed in a weighed, screw-capped vial, reweighed, and diluted with 1 M piperidine to a weight to volume ratio of 1:10. The tissue was digested at about 56° C for 24 hours, after which 0.5—1 ml of the digest was placed in a planchet, slowly evaporated to dryness (about 12 hours), and the radioactivity determined. Previous experiments have shown that all radioactivity is recovered by this procedure.

Electrolytes. Plasma and CSF were analyzed for Na, K and Cl as follows: CSF and some plasma samples were extracted and diluted in 1 N HNO_3 with 15 mEq/l LiCl for determina-

tions. The plasma obtained after centrifugation was measured and diluted in 15 mEq/l of LiCl at a volume ratio of 1:200. Analysis for Na and K in each sample was made by means of internal standard flame photometry (Instrumentation Laboratory, Inc.). The Cl concentration of the fluids was measured by the electrometric titration method of COTLOVE *et al.* (1958).

Calculations. Concentration ratios of CSF/plasma water and brain/plasma water for ^{14}C-inulin and ^{14}C-sucrose were calculated and time-uptake curves plotted for the rats of various ages. All uptake curves were resolved into components by the method described by SOLOMON (1949) and applied to brain by WOODBURY (1958).

Results

Figure 1 is a semilogarithmic plot of the time-uptake curves for ^{14}C-inulin in CSF and brain of 4-day prenatal and 3-, 9-, and 16-day postnatal rats. Twentyfour hour uptake ratios for a litter of newborn and a group of 26-day old rats also are

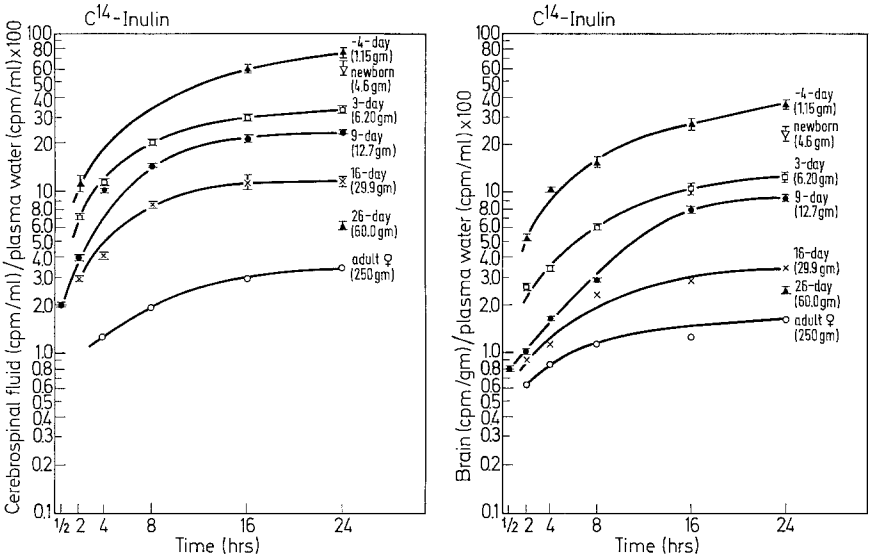

Fig. 1. *The simultaneous uptake of ^{14}C-inulin into cerebral cortex (called brain in subsequent figures) and cerebrospinal fluid (CSF) of rats during maturation, as a function of the time after injection of the inulin.*

Ordinate on the left: $\left[\frac{\text{cerebrospinal fluid } ^{14}\text{C-inulin concentration (cpm/ml)}}{\text{Plasma water } ^{14}\text{C-inulin concentration (cpm/ml)}}\right] \times 100$;

on the right: $\left[\frac{\text{Brain } ^{14}\text{C-inulin concentration (cpm/gm)}}{\text{Plasma water } ^{14}\text{C-inulin concentration (cpm/ml)}}\right] \times 100$;

both ratios yielding values for spaces (in %). The abscissa in both graphs is time in hours. All the animals were bilaterally nephrectomized for 24 hours. The vertical bracketed lines at each point represent the standard errors of the mean value represented by the point. In the case of the 16-day old animals, for the brain/plasma water ratio shown on the right side, the standard error values are not shown because they are smaller than the size of the symbols. See text for discussion

plotted, as are the points and uptake curves for the mothers of the prenatal and newborn animals. Each point represents the average of values obtained for 6 or more animals except in the case of the mother rats. The curves were fitted to the points by eye.

With increasing age of the animals there is a progressive decrease in the measured inulin ratios of both compartments. Most of the curves indicate that peak inulin levels were achieved at 24 hours in the CSF and brain. Each of the curves was analyzed for the number of components of uptake as described in Methods. All were found to be one-component curves. It should be noted that in the prenatal and newborn animals the inulin crossed the placental barrier since it was injected intraperitoneally into the mother rats.

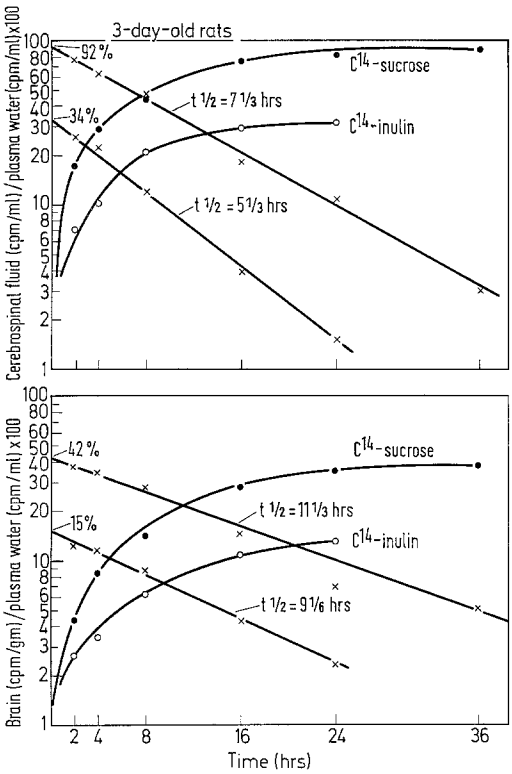

Fig. 2. *The uptake of ^{14}C-sucrose and ^{14}C-inulin simultaneously into brain and CSF of 3-day old rats as a function of time after administration.* The upper portion of the figure represents penetration of the isotopes into CSF and the lower portion their uptake into brain. The ordinates are the [CSF/plasma water] and [brain/plasma water] ratios × 100 and the abscissa is the time in hours after intraperitoneal injection of the indicator substances. All the animals receiving inulin were bilaterally nephrectomized for 24 hours; the rats receiving sucrose, for 36 hours. The curves have been resolved by graphical analysis into their components (SOLOMON 1949). The half lives of the components ($t\ ^1/_2$) in hours are indicated on the resolved lines. Only one component is present. The intercept of each curve, which represents the equilibrium volume (in %) of distribution of the indicator substance, is shown on each component curve at its junction with the ordinate at zero time. See text for further explanation

In Figs. 2 and 3 the uptake curves for labeled inulin are compared with those for labeled sucrose for the 3- and 16-day old rats. The curves are resolved into their components by graphical analysis. Although both curves of each age group resolved into only one component, sucrose consistently measures larger "spaces" than does inulin.

In Fig. 4 the uptake of inulin into the extracellular space of muscle is shown for the 3-, 9-, and 16-day old animals, and the uptake of sucrose into the extracellular space of muscle in 3-day old animals. The corresponding spaces at equilibrium were 50, 38, and 30%. Labeled sucrose is taken up more rapidly in the 3-day old animals, but reaches a similar level by 8 hours. Sucrose (molecular weight, 342) is a much smaller molecule than inulin (molecular weight, about 5000), and therefore penetrates more readily into the extracellular space of tissues. These curves were included to show uptake of these substances by a non-neural tissue.

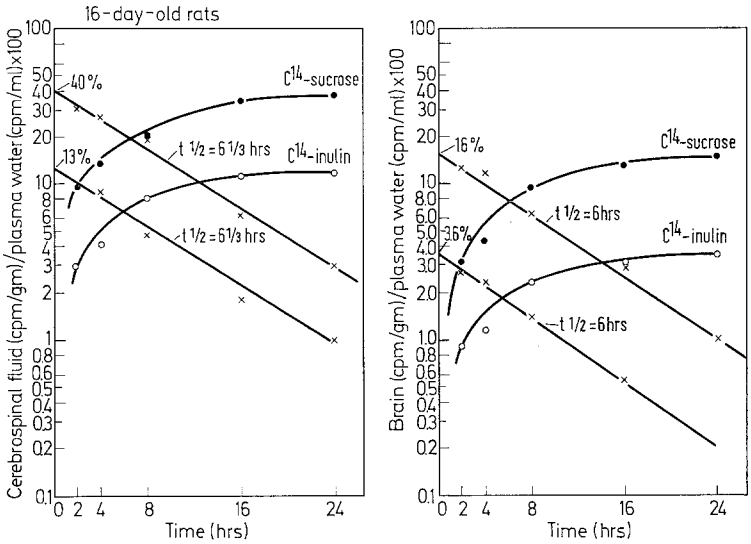

Fig. 3. *The uptake of ^{14}C-sucrose and ^{14}C-inulin simultaneously into brain and CSF of 16-day old rats as a function of time after administration.* The left-hand portion of the figure represents penetration of the isotopes into CSF and the right-hand portion the uptake into brain. The ordinates are the [CSF/plasma water] and [brain/plasma water] ratios × 100 and the abscissa is the time in hours after intraperitoneal injection of the indicator substances. All the rats were bilaterally nephrectomized for 24 hours. The curves have been resolved by graphical analysis into their components (Solomon 1949). The half lives of the components ($t^1/_2$) in hours are indicated on the resolved lines. Only one component is present. The intercept of each curve, which represents the equilibrium volume (in %) of distribution of the indicator substance, is shown on each component curve at its junction with the ordinate at zero time. See text for further explanation

In Table 1 the 24-hour-uptake ratios are tabulated for inulin and sucrose for all ages of animals studied. The brain/CSF ratios were obtained by dividing the CSF/plasma ratios into the brain/plasma ratios. Also shown are the half-lives of the single component of the uptake curves for the animals in which a full curve was obtained. For the remainder only 24-hour-uptake ratios were measured.

Since significant degrees of azotemia may exist in rats after 6 hours of nephrectomy (Fishman and Raskin 1967), experiments were done to compare the effect of duration of nephrectomy on the inulin ratios measured in 9-day-old rats. Eight-hour brain uptakes were 3.5, 3.4 and 3.0% among rats which were nephrectomized 8, 16, and 24 hours respectively; corresponding CSF uptakes in the same animals also did not differ appreciably (13.2, 16.0 and 12.8%, respectively). Similarly,

16-hour-uptake ratios for both brain and CSF among rats nephrectomized either 16 or 24 hours were not significantly different (4.4 and 15.8 versus 5.4 and 18.6). Hence, in this study, nephrectomy did not seem to alter the tissue "spaces" measured by inulin. In the study of FISHMAN and RASKIN (1967), although

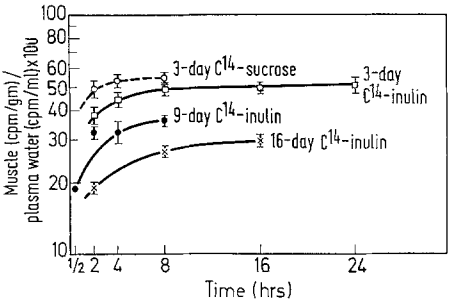

Fig. 4. *Comparison of the uptake of ^{14}C-inulin into skeletal muscle of 3-, 9-, and 16-day old rats at different times after intraperitoneal injection and of ^{14}C-sucrose in 3-day old rats.* All the rats were bilaterally nephrectomized for 24 hours. The ordinate is the volume of distribution of inulin measured as the [muscle/plasma water] ratio × 100 (inulin or sucrose space in per cent), and the abscissa is time in hours after injection. Unlike the brain, the muscle spaces measured by ^{14}C-sucrose and ^{14}C-inulin in 3-day old rats are not significantly different at equilibrium, although sucrose more rapidly reaches the equilibrium value of about 52%. The equilibrium value for the muscle of 9-day old rats is about 37%; for 16-day old animals, about 30%. The inulin space of adult muscle is about 8% (WOODBURY 1965a). Thus the extracellular space of skeletal muscle, like that of brain, decreases markedly during maturation. See text for further discussion

nephrectomy appeared to change the rate of entry of sucrose into brain, it did not alter the actual levels achieved after 24 hours in normal versus "uremic" rats.

In Fig. 5 is shown a plot of the 24-hour brain/plasma and brain/CSF ratios for inulin and sucrose. The values for the points are those shown in Table 1, whereas the curves shown were fitted to the points by eye. In the 4-day prenatal animals the brain/plasma and brain/CSF ratios for inulin approach each other, an indication that at this age the two ratios measure nearly the same space. Although both decrease with maturation, the two ratios increasingly diverge. At 16 days of age the brain/CSF ratio reaches a minimum and increases thereafter, an indication of another change in the distribution of inulin between brain and CSF. The curve is extrapolated as a dotted line downward to a value of 13.5% (indicated by an asterisk). The source of this value and the reason for the extrapolation are discussed in the next section.

That sucrose measures a larger brain space than inulin is indicated by the fact that at all ages the brain/CSF ratios for sucrose are greater than those for inulin. This could result from sucrose or some impurity in the radioactive sucrose entering cellular elements in the brain. This possibility also will be discussed in the following section.

In Table 2 the values obtained for Na, K and Cl in the CSF and plasma are shown for both nephrectomized and non-nephrectomized rats of various ages and are plotted as ratios in Fig. 6. For both the prenatal and newborn animals the mother rats were nephrectomized, but with the difference that the prenatal rats

remained attached to their placentas at the time of obtaining samples. Samples from the newborn rats were not taken until several hours after birth. It is apparent from this graph that the Na and Cl concentration ratios in intact rats tend to increase slightly with age during early life and then level out whereas the K concentration ratio decreases with age. As a result of slight decreases of plasma Na and Cl during early life (see Table 2), the ratios of the CSF/plasma Na and Cl

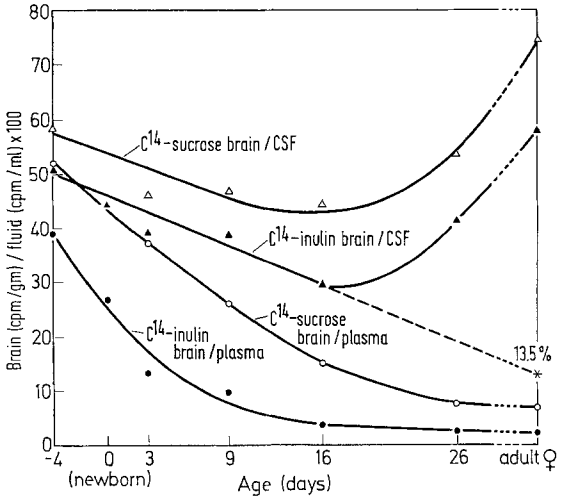

Fig. 5. *Relation between the brain to fluid (CSF or plasma water) ratio* × 100 *(space in %) of* ^{14}*C-inulin and* ^{14}*C-sucrose and the age in days after or before birth of rats.* The asterisk (*) shown at the end of the extrapolated line (dotted) representing an extension of the ^{14}C-inulin brain/CSF ratio is at a value of 13.5%. This value was obtained in rats by WOODWARD *et al.* (1967) by ventriculocisternal perfusion of ^{14}C-inulin until equilibrium was attained in the brain. It represents the true extracellular space of the adult rat brain. See text for further discussion

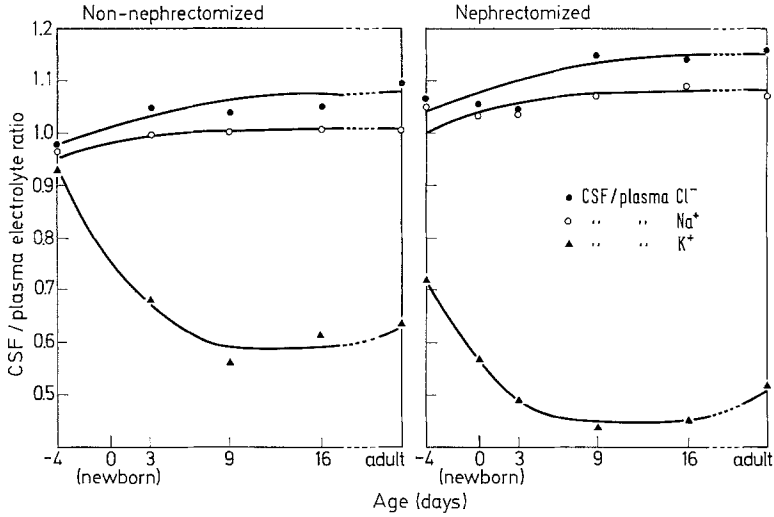

Fig. 6. *Plot of CSF/plasma electrolyte concentration ratios for rats of various ages.* The ratios were calculated from the mean values shown in Table 2. The curves were fitted to the points by eye

Table 1. *24-Hour uptake ratios and half-lives of the uptake curves for ^{14}C-inulin and ^{14}C-sucrose*

AGE	^{14}C-INULIN			^{14}C-SUCROSE		
	Brain /Plasma %	CSF/Plasma %	Brain/CSF %	Brain/Plasma %	CSF/Plasma %	Brain/CSF %
4-day-prenatal . .	38.8 (9 hrs)*	76.2 (9 hrs)	50.9	51.8	88.3	58.6
Newborn	26.7	59.8	44.6	—	—	—
3-day-old	13.2 ($9\text{-}^1/_6$ hrs)	33.3 ($5\text{-}^1/_3$ hrs)	39.6	37.2 ($11\text{-}^1/_3$ hrs)	81.3 ($7\text{-}^1/_3$ hrs)	45.7
9-day-old	9.5 ($6\text{-}^2/_3$ hrs)	24.2 ($5\text{-}^2/_3$ hrs)	39.3	25.8	55.1	46.8
16-day-old	3.5 (6 hrs)	11.8 ($6\text{-}^1/_3$ hrs)	29.6	14.9 (6 hrs)	33.3 ($6\text{-}^1/_3$ hrs)	44.7
26-day-old	2.6	6.3	41.3	7.7	14.4	53.5
Adult	1.9	3.4	58.0	6.8	9.4	75.0

* Values in parentheses indicate half-lives of the single components of the uptake curves.

Table 2. *Mean values ($\pm$ s. e.) of CSF and plasma electrolytes for nephrectomized and non-nephrectomized rats of various ages*

Age	Condition	CSF			Plasma		
		Na	K	Cl	Na	K	Cl
		mEq/l			mEq/l		
4-day-prenatal . . .	Non-nephrex	143.8 $\pm$ 8.1	6.25 $\pm$ 0.29	108.9 $\pm$ 3.6	148.5 $\pm$ 1.6	6.69 $\pm$ 0.42	112.5 $\pm$ 2.9
	Nephrex	148.4 $\pm$ 2.1	8.32 $\pm$ 0.45	120.7 $\pm$ 3.8	140.3 $\pm$ 1.9	11.6 $\pm$ 1.1	113.2 $\pm$ 2.0
Newborn.	See text	147.5 $\pm$ 3.4	4.54 $\pm$ 0.18	116.8 $\pm$ 3.7	143.8 $\pm$ 1.2	7.98 $\pm$ 0.87	110.3 $\pm$ 2.1
3-day-old	Non-nephrex	148.5 $\pm$ 2.1	4.50 $\pm$ 0.43	113.8 $\pm$ 1.5	150.3 $\pm$ 2.0	6.64 $\pm$ 0.76	108.0 $\pm$ 3.0
	Nephrex	149.6 $\pm$ 2.4	4.00 $\pm$ 0.20	111.8 $\pm$ 2.0	145.6 $\pm$ 1.5	8.18 $\pm$ 0.52	107.8 $\pm$ 2.7
9-day-old	Non-nephrex	147.5 $\pm$ 2.1	4.01 $\pm$ 0.19	123.1 $\pm$ 2.2	146.3 $\pm$ 1.3	7.34 $\pm$ 0.34	118.9 $\pm$ 2.1
	Nephrex	150.3 $\pm$ 0.9	4.36 $\pm$ 0.15	119.5 $\pm$ 1.2	141.0 $\pm$ 1.6	9.85 $\pm$ 0.72	103.5 $\pm$ 1.4
16-day-old	Non-nephrex	151.1 $\pm$ 1.2	4.49 $\pm$ 0.32	120.5 $\pm$ 1.6	148.6 $\pm$ 4.2	7.28 $\pm$ 0.46	116.3 $\pm$ 2.5
	Nephrex	158.5 $\pm$ 1.7	4.86 $\pm$ 0.27	122.5 $\pm$ 0.9	144.9 $\pm$ 2.2	10.7 $\pm$ 0.3	107.1 $\pm$ 2.4
Adult	Non-nephrex	148.0 $\pm$ 0.8	2.86 $\pm$ 0.16	117.9 $\pm$ 1.2	145.6 $\pm$ 1.0	4.49 $\pm$ 0.24	106.9 $\pm$ 1.3
	Nephrex	148.8 $\pm$ 0.8	3.39 $\pm$ 0.17	119.2 $\pm$ 1.3	143.5 $\pm$ 1.2	6.24 $\pm$ 0.22	102.5 $\pm$ 2.5

increase. Plasma K decreases generally throughout development and the CSF/plasma K reaches a minimum at about 9 days of age. In nephrectomized animals the electrolyte ratios change with age in the same direction as they do in intact animals but the values are greater for Na and Cl and smaller for K at all time periods. These changes will be discussed later.

In one 6-day prenatal rat not shown in the graph, the CSF electrolytes were found to have almost the same concentrations as the mother's plasma, indicating that at this age in development, the fetal CSF is still an ultrafiltrate of the mother's plasma. Between the 6-day prenatal and the 9-day postnatal animals, the ratios change progressively.

Discussion

Factors Influencing Measurements of Brain Extracellular Space. A plasma solute which distributes in the brain extracellular space could provide a measure of the size of that space by either of two ratios, the brain/plasma or the brain/CSF. If the substance equilibrated readily between plasma and interstitial fluid of the brain, the brain/plasma concentration ratio should measure the size of the space. If the substance distributed readily between brain interstitial fluid and cerebrospinal fluid, the brain/CSF concentration ratio should measure the extracellular space.

It is clear that in adult animals neither of these ratios provides a true measure of the extracellular space of the brain when inulin or sucrose injected into the plasma is used as the measuring substance. In adult rats, for example, REED and WOODBURY (1963) found an inulin brain/plasma ratio of only 1.5—2% and an early sucrose ratio of 4%. Not only do these ratios seem too low for the functional extracellular space, but they are not in agreement as to its size. Conversely, the brain/CSF ratios for these substances are too high, approaching 100% in adult rats.

Several mechanisms may distort equilibration between the three fluids and thus account for failure to validate the theoretical relationships between them. In the case of the brain/plasma ratio, failure to measure the brain extracellular space with inulin or sucrose probably results from the "sink effect" of the CSF (DAVSON 1963) combined with slow penetration of these substances across the cerebral capillaries into the adult brain. In young animals in which the choroid plexuses have not matured (a 6-day prenatal rat in our experiments), the CSF is an ultrafiltrate of the plasma produced by the action of the heart. The onset of CSF secretion and increased CSF flow through the arachnoid villi would initiate the "sink effect" by increasing the gradient for substances diffusing from brain into CSF. In fetal pigs the active secretion by the choroid plexuses was found to begin with the middle third of gestation, as judged by the distribution of Na, Cl and urea between CSF and plasma (FLEXNER 1938; FLEXNER and STIEHLER 1938). In a growth interval of only 3 days, these solutes attained distribution ratios like those described for adult animals. Consequently, during this period, the CSF was believed to have changed from an ultrafiltrate to a secretion.

The effect of CSF drainage in reducing accumulation of substances in the brain would be further magnified by any barrier formation to their penetration across the brain capillaries, since rate of entry into the brain would decrease while the rate of exit in the CSF remained the same.

Similarly, failure to measure the brain extracellular space by the brain/CSF ratio might result from formation of a barrier between the brain and CSF which slowed the rate of equilibration between brain interstitial fluid and CSF. In this case, CSF flow would exaggerate the difference in concentrations in the two fluids, tending to increase further the brain/CSF ratio. However, increased CSF flow would not be expected to affect the equilibration in any significant way as long as there was no barrier to diffusion between brain and CSF.

Two other possible influences must be considered. If the extracellular space of the brain decreased during its maturation, then both the brain/plasma and the brain/CSF ratios would decrease. Both anatomic and functional studies indicate that the brain extracellular space decreases during development (BRIZZEE and JACOBS 1959a, 1959b; VERNADAKIS and WOODBURY 1965; KARLSSON 1967). Conversely, if the substances used were not confined strictly to the extracellular fluid but instead penetrated cellular elements to a greater or lesser extent, then both ratios would increase but would fail accurately to measure the extracellular space. Although this situation would increase the ratios, the intracellular location of the substance might be difficult to detect simply by its measurement in the total brain, plasma and CSF, and other approaches would be necessary.

Many factors thus can influence the uptake and accumulation of substances in the extracellular fluids of the brain. The purpose of the present study was to define more clearly these factors and their temporal manifestations in developing rats. The progressive decrease in the per cent concentration in both CSF and brain with age of the animals (Figs. 1 and 2; Table 1) could have resulted from any of the several mechanisms discussed above. Comparisons between the brain/plasma and the brain/CSF ratios, combined with certain other analysis, suggest which mechanisms are operative at various stages of development.

Temporal Changes of Concentration Ratios. The best approach to a consideration of our data is temporal (see Fig. 5). In the 4-day prenatal rats the 24 hour brain/plasma and brain/CSF inulin ratios approach one another, an indication that at this period of development the two ratios measure approximately the same space. With no barriers to diffusion and no "sink effect" of the CSF the two ratios in the steady-state should measure the same space in the brain. The fact that the ratios have already diverged slightly at this age suggests that one and probably both of these mechanisms have already become effective. Increasing CSF flow is indicated during this period by the CSF/plasma electrolyte ratios shown in Fig. 6 (derived from Table 2). The increasing Na and decreasing K ratios suggest active transport of these ions with associated CSF formation and bulk flow through the arachnoid villi. This is the case because CSF flow results from the active transport of Na and probably Cl into the CSF at the choroid plexus, the evidence for which is summarized by WOODBURY (1965b). In one 6-day prenatal animal the CSF electrolytes were found to exist in the same concentrations as those in the mother's plasma, a result that suggests CSF flow has not begun at this age. However, the leveling out of the ratios after 3 days postnatal suggests that the secretory mechanisms are mature. This does not necessarily mean that flow is maximal, since the volume of secretory cells might still increase with age.

Although there is a proportionately greater decrease of the brain/plasma ratio, both ratios decrease during this period. Although the maximal rate of CSF flow

in the rat is not known, presumably the brain/CSF ratio would be unaffected by increasing CSF flow as long as the flow rate did not greatly exceed the rate of equilibration between interstitial fluid and CSF. For electrolytes and smaller solutes the latter should be fairly rapid. Thus, barring the development of a barrier between the CSF and brain, the brain/CSF ratio should estimate the brain extracellular space. The decrease in this ratio then would indicate that the size of the extracellular space drecreases with age. This conclusion is supported by other studies, previously mentioned, one of which (VERNADAKIS and WOODBURY 1965) calculates an interstitial component of 39% in 8-day old rats, a value in agreement with the ^{14}C-inulin brain/CSF space shown in Fig. 5 for 8-day old rats. The same study further suggests that the volume of glial cells increases at the expense of the extracellular volume.

On the basis of the brain/CSF ratio, the extracellular space decreases until 16 days of age. Between the 16th and 26th day the ratio reverses and on the 26th day it is already above the value at 16 days (Fig. 5). However, it is unlikely that the extracellular space again increases since recent work, in which simultaneous perfusion of plasma and ventricular system was employed, indicates that in the rat the inulin space is 13.5% of the brain volume (WOODWARD *et al.* 1967). Thus, the true extracellular space would continue to decrease, as shown by the dotted line in the figure. This discrepancy between the latter observation and the space measured with inulin injected into the plasma could result from either penetration of inulin into cellular elements or development of a barrier to inulin across the ependyma between brain and CSF. Since the inulin uptake curves in adult rats resolve into only one component and since data from other tissues show that inulin is confined to the extracellular space, it appears that the best explanation is an ependymal barrier formation to inulin as the animals mature.

On the other hand, the ratios for sucrose consistently measure larger spaces for the two compartments in all ages. This fact suggests that sucrose penetrates a space that inulin does not. In this regard, sucrose has been noted to distribute in larger volume than does inulin in cerebral cortical slices (PAPPIUS 1965). In these studies sucrose was considered to penetrate an intracellular compartment, *viz.*, the glial cells. Although the uptake curves shown for sucrose in Fig. 2 resolve into one component, two components have been found in adult rats (REED and WOODBURY 1963). This would suggest that in the younger rats sucrose must penetrate the extra space or compartment at about the same rate that it does the extracellular space.

It should be mentioned, however, that no attempt was made to completely exclude radioisotope contamination (NICHOLLS and WOLFE 1967) and the possibility exists that the larger sucrose ratios are influenced by contaminants or products of metabolism. Comparisons with previous studies suggest little or no variability in sucrose results, however. That contaminants are not the cause of the larger sucrose spaces in brain is indicated by the fact that the sucrose and inulin spaces of muscle are equal. If contaminants that could penetrate cells were present the sucrose space of muscle, like that of brain, should be larger than the inulin space.

Some of the mechanisms which underlie decreased inulin concentration in the brain and CSF of developing rats are shown schematically in Fig. 7. In the very young animal the flow of CSF through the arachnoid villi is probably sluggish

but increases as the choroid plexus matures. There is a larger extracellular space in the brains of young animals with little or no barrier to entry from the plasma. Increasing CSF flow, barrier formation between blood and brain, and decreasing extracellular space all contribute to decreased concentration in the brain and a

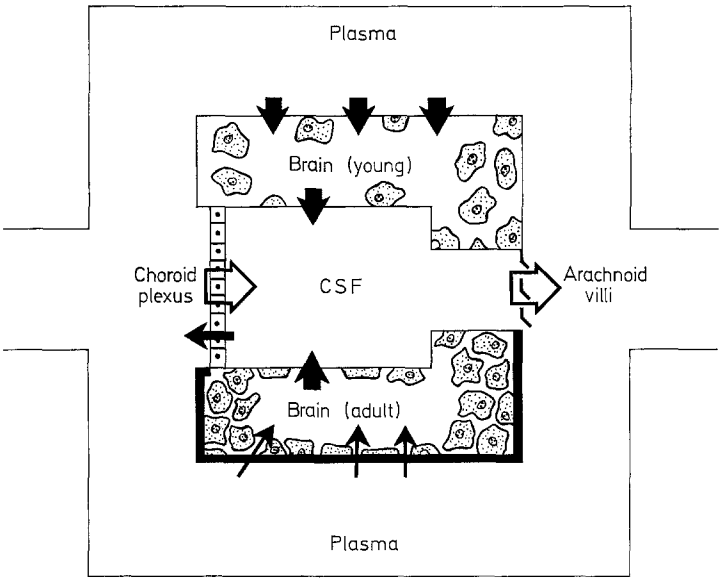

Fig. 7. *Schematic drawing of the various factors that regulate the exchange of substances between plasma, brain, and cerebrospinal fluid in young as compared with adult rats.* The filled arrows indicate the movement of solutes and the open arrows the flow of cerebrospinal fluid. The width of the arrows is proportional to the rate of flow of the solutes across the various boundaries. The thicker boundary line at the brain-plasma interface of the adults as compared with the young is an indication of the greater development of the "blood-brain barrier" in the older group. See text for further discussion

lowering of the brain/plasma ratio for a substance in the interstitial space. However, the brain/CSF ratio continues to estimate the extracellular space as long as no barrier forms across the ependyma between brain and CSF. The upturn in the brain/CSF ratio in animals older than 16 days of age indicates that the rate of movement of inulin across the ependyma decreases with age since there is no convincing evidence that this substance can enter cells (Woodbury 1965a). It is clear, therefore, that neither ratio (brain/plasma or brain/CSF) gives a true measure of the extracellular space of the brain after this age period.

Effects on CSF Electrolytes of Age and Nephrectomy. The changes in CSF electrolytes with age require some discussion (Fig. 6). In the nonnephrectomized control animals the ratios of CSF/plasma Na and Cl concentrations slightly increase with age before 9 days whereas the ratio of CSF/plasma K decreases with age until 9 days, the lowest value being reached at this time. Nephrectomy results in an increase in the Na and Cl and a decrease in the CSF/plasma K ratios at each age period, but highest and lowest values are still reached by the same age. The values for the CSF/plasma ratios for Na and K in adult rats are out of equilibrium with the observed potential difference between CSF and plasma as measured by

WELCH and SADLER (1965) for the rabbit, HELD *et al.* (1964) for the dog and goat and the value of +3.3 mV determined for the rat by KJALLQUIST and SIESJÖ (1967). This is evidence that the transport of these ions across the choroid plexus is active (see discussion of the evidence that Na and possibly Cl are actively transported across the choroid plexus and that CSF flow results from their active transport in WOODBURY 1965b). The fact that these ratios are close to the Donnan distribution values in the prenatal rats also suggests that active transport of Na or K has not developed as yet and that the potential difference across the choroid plexus is near zero. The fact that the CSF/plasma ratio of Na changes in the opposite direction from that of K with age and with nephrectomy also suggests that these ions are transported in opposite directions across the choroid plexus probably (as is the case in other tissues) as a Na for K coupled pump in the cell membrane on the CSF side of the epithelial cells making up the choroid plexus.

The changes in the CSF/plasma ratios with nephrectomy are of interest since it has been demonstrated in muscle (FOZZARD and KIPNIS 1965; WOODBURY 1965a) and other tissues (WOODBURY, unpublished observations) that an elevated plasma K concentration stimulates active transport of the Na for K coupled pump, resulting in a decreased concentration of Na in cells. The same event appears to occur in the CSF. The elevated plasma K concentration, by depolarization of the cell membrane, stimulates the active transport of the Na for K coupled pump in the choroid plexus cells and thereby increases the CSF/plasma ratio of Na and decreases that of K. In the 4-day prenatal rats where the active transport process is probably still sluggish, a 33% increase in CSF K concentration in response to a 71% increase in plasma K concentration following nephrectomy is much greater than in 16-day old rats in which the Na for K coupled pump is fully developed; in the latter group the increase in plasma K concentration following nephrectomy is 47% and the concomitant increase in CSF K concentration is only 8% (see Table 2). It is likely, therefore, that the maintenance of the CSF K level at near normal values in response to an elevated plasma K concentration reported by many investigators (BEKAERT and DEMEESTER 1954; AMES *et al.* 1965; BRADBURY and DAVSON 1965; CSERR 1965; KATZMAN *et al.* 1965; CSERR and RALL 1967) can be explained by activation of the Na-K pump across the choroid plexus as a result of the depolarization produced by the elevation of the K concentration in plasma, as described by WOODBURY (1965a) and FOZZARD and KIPNIS (1965). This appears to be a regulatory mechanism for maintaining a constant K concentration in the CSF, which is probably also the fluid that surrounds the neurons and glia as extracellular fluid.

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