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KINETICS OF MOVEMENT OF IODIDE, SUCROSE, INULIN AND RADIO-IODINATED SERUM ALBUMIN IN THE CENTRAL NERVOUS SYSTEM AND CEREBRO-SPINAL FLUID OF THE RAT

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Since Ehrlich reported in 1885 that certain dyes that stain most tissues of the body were excluded from the central nervous system, a large volume of information has accumulated concerning the selective exclusion of many substances from the central nervous system (C.N.S.). The term 'blood–brain barrier' has been coined to describe this phenomenon. Our understanding of the mechanisms by which many substances are excluded from the nervous system, others are severely limited in their entry and distribution, and some are permitted to enter relatively freely is very incomplete, though more than 75 years have elapsed since Ehrlich's observations.

An understanding of the physiological significance of the blood–brain barrier and its relation to brain function would appear to be dependent upon an elucidation of the physiological compartments of the C.N.S. and the interchange of substances between these compartments; as yet there is no unequivocal evidence that the blood–brain barrier is associated with any specific anatomical structure. The measurement of only one or two isolated variables such as the brain:plasma or cerebrospinal-fluid (c.s.f.):plasma ratio has proved inadequate to elucidate this complex problem, since the significance of a given quantity is determined primarily by the simultaneous absolute values in the other compartments as well as the rates of exchange between compartments. Thus, it would seem desirable to determine in the C.N.S. (blood, c.s.f. and brain) simultaneous efflux and influx rates and concentrations of a variety of substances which, in other tissues, are known to distribute in defined physiological spaces (extracellular fluid (E.C.F.), blood, etc.). Also, with the information that is available on the concentration of electrolytes in brain tissue, c.s.f. and plasma, and with the membrane potential measurements that have been

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obtained from neurones and glial cells, it should be possible by use of the Nernst equation to calculate the concentration of electrolytes in the neurones, glial cells, and extracellular space if the volumes of at least two of these spaces are known. A knowledge of the relative sizes of the various brain compartments (neuronal, glial, and extracellular) and the electrolyte concentrations in each could contribute materially to an understanding of the roles of the various cell types in the function of the brain. The following experiments were performed as part of a kinetic study of the blood–brain barrier and in search for a measure of the relative size of one or more of the compartments of the brain.

Since iodide (Wallace & Brodie, 1937, 1939, 1940*a*, *b*; Gildea & Man, 1943; Greenberg, Aird, Boelter, Campbell, Cohn & Muryama, 1943; Davson, 1955; Davson & Spaziana, 1959), sucrose (Hubbard & Zoll, 1949; Deane, Schreiner & Robertson, 1951; Gamble, Robertson, Hannigan, Foster & Farr, 1953; Davson & Matchett, 1953; Davson, 1955; Peterson, O'Toole, Kirkindall & Kemp-Thorne, 1959), inulin (McLennan, 1957; Morrison, 1959; Rothman, Freireich, Gaskins, Patlak & Rall, 1961), and serum albumin (Fishman, 1953; Rozdilsky & Olszewski, 1957; Van Wart, Dupont & Kraintz, 1960; Lee & Olszewski, 1960; Brown, 1961) have been used in various space measurements and barrier studies, and since they are relatively non-toxic substances, representing both charged and uncharged molecules and ions that have markedly different molecular weights (iodide 127, sucrose 342, inulin 3000–4000, albumin 69,000), they were selected for the present studies of transport and barrier mechanisms.

In this investigation the tracers were administered to rats intraperitoneally (I.P.), intravenously (I.V.), or intracisternally (I.C.). The distribution of each tracer in the c.s.f., brain cortex (brain), muscle, and plasma water and its movement from one compartment to another were studied over relatively long periods in order to obtain information that might elucidate the size of the extracellular space of the brain, the barriers to the movement of the substance, and the pathway or pathways of influx and efflux in the C.N.S.

METHODS

Male Sprague-Dawley rats weighing 200–250 g were used as experimental animals. Under light ether anaesthesia a dorsal mid-line skin incision was made and the kidneys were decapsulated and the pedicles ligated by a dorsolateral approach. Following this functional nephrectomy the muscle wall was sutured and the skin incision closed with wound clips. Food and water were provided *ad libitum* throughout the experiment.

The I.C. injections were facilitated by placing the lightly etherized rat in a special holder, in which anaesthesia could be maintained and the head flexed to any desired degree and fixed in that position. This holder has been described in some detail previously (Reed & Woodbury, 1962). The I.C. injections were made through a 22-gauge short-bevel needle which accommodated two 0·25 ml. syringes, one being empty and the other containing

0·1 ml. of the fluid to be injected. The needle was introduced into the cisterna magna through the foramen magnum. Slightly more than 0·1 ml. of c.s.f. was withdrawn from the cisterna magna into the empty syringe, and the solution from the other syringe was injected and flushed in with the c.s.f. in excess of 0·1 ml. that was initially withdrawn. Thus, when the needle was withdrawn the volume of c.s.f. removed equalled the volume of solution added.

All injections were timed so that the animal had been nephrectomized for 24 hr when the tissue samples were taken. Where experiments were to extend over 24 hr or longer the injection was made immediately following nephrectomy.

At the times after injection specified for each experiment (with the animal under the same conditions as previously described for injection), a 50 μl. sample of c.s.f. was withdrawn from the cisterna magna into a graduated micropipette. Immediately after removal of the c.s.f. as much blood as possible was withdrawn from the abdominal aorta into a heparinized syringe, the brain was removed, the meninges were separated from the cerebral hemispheres, and the cortical tissue was removed and carefully blotted. A sample of muscle from a hind limb was excised and blotted, and the superficial fat was removed. The time from the withdrawal of the c.s.f. to the withdrawal of the blood was about 15–20 sec.

The tissues were prepared for analysis as follows. Fifty microlitres of c.s.f. was transferred from the graduated micropipette to a planchet and 1 ml. distilled water added. After agitation to insure mixing, the c.s.f. was evaporated to dryness for counting the radioactivity. One half of the cortex was dried at 105° C to a constant weight and the water content was calculated from the difference between wet and dry weights. The other half of the cortex was placed in a tared, screw-capped tube containing 3 ml. of 1 M piperidine, weighed, and digested at 55–60° C for 24 hr. 1 ml. of digest was pipetted into a planchet and evaporated to dryness. Samples of muscle were treated like brain. 0·5 ml. samples of plasma were dried and counted. In the case of plasma samples containing iodide the total radioactivity of the sample was corrected by the amount of activity associated with the protein fraction precipitated by 10% trichloroacetic acid. This correction was usually less than 1% of the activity of the sample, which agrees well with the report of George & Sollich (1959).

The radioactivity of ground samples of the dried tissues was compared with that of the digested tissues from the same animal; the two procedures gave the same results. All samples were counted for at least 4096 counts in a Geiger–Müller counter. The radioactivity of all tissues was corrected for absorption and, within each experiment, to the same injected dose.

In view of the presence of a slight amount of residual blood in brain and muscle and of the possible effect of an I.C. injection on the distribution of a tracer in the brain, an attempt was made to quantify these possible sources of error. To determine the quantity of blood remaining in the tissues, ^{51}Cr-chromate-labelled erythrocytes and ^{131}I-labelled serum albumin were used according to the method of Sterling & Gray (1950). To assess the effect of the I.C. injections, 104 rats, divided into 13 groups of 8 animals each, were injected I.P. with 10 μc of Na ^{131}I and 4 μc of uniformly labelled ^{14}C-sucrose in a solution made isotonic with NaCl and in a volume of 0·1 ml. In addition, 4 of the animals in each group were injected I.C. (sham injection) with 0·1 ml. isotonic NaCl solution, as explained above. The 4 remaining animals in each group of 8 animals were the controls for the 4 rats injected I.C. A group of 8 animals was killed at each of the following time periods: 5, 10, 20 and 30 min and 1, 2, 4, 8, 16, 24, 30, 40 and 43 hr. The tissues were sampled and the radioactivity was determined according to the methods previously described.

In all experiments in which ^{131}I and ^{14}C were present in the same tissues, the radioactivity of each was determined by counting the iodide with the ^{14}C filtered out and by counting the ^{14}C after the ^{131}I had, for all practical purposes, completely decayed (more than 8 times the iodide half-life of 8 days). The information concerning dose, injection times, and number of animals used, as they relate to the experiment with each tracer, is given below.

Iodide

Two series of experiments were performed. In one series 80 nephrectomized rats were divided into 10 groups of 8 animals each. One half of the animals in each of the 10 groups was injected I.C. with 10 μc of Na ^{131}I (carrier-free) and 4 μc of ^{14}C-labelled sucrose (0·008 mg/μc) in a solution made isotonic with NaCl and in a volume of 0·1 ml. The other half received the same dose of isotopes injected I.P. One group was killed at each of the following times after injection: 30 min and 1, 2, 4, 8, 16, 24, 30, 40 and 43 hr. The second series was carried out in the same manner as that just described, except that there were 5 groups of 8 animals each, the injected solution did not contain ^{14}C-labelled sucrose, and the times from injection until the tissues were sampled were 2, 5, 10, 20 and 30 min. Also, a catheter was placed in the abdominal aorta to permit the simultaneous withdrawal of blood and c.s.f.

Sucrose

In one series of experiments sucrose alone was administered to 72 rats divided into 8 groups of 9 animals each. Of each group of 9 animals 4 were injected I.P. with 2 μc of ^{14}C-sucrose in a volume of 0·2 ml. of isotonic NaCl solution. The remaining 5 animals in each group received 1 μc of ^{14}C-sucrose in 0·1 ml. of NaCl solution I.C. A group was killed at 10 and 30 min and 1, 2, 4, 8, 16 and 24 hr after injection. The second series consisted of 80 animals divided into 10 groups of 8 animals each, in which Na ^{131}I-iodide and ^{14}C-sucrose were administered simultaneously. One half of each group received 10 μc of ^{131}I and 4 μc of ^{14}C-sucrose in 0·1 ml. of NaCl solution I.C. and the other half received the same dose I.P. One group was killed at each of the following times after injection: 30 min and 1, 2, 4, 8, 16, 24, 30, 40 and 43 hr.

Inulin

Carboxyl-^{14}C-inulin (2 μc/0·1 ml.) in isotonic NaCl solution was administered either via the external jugular vein, or I.C., to each rat. The 64 animals were divided into 8 groups of 8 animals each; one half of each group was injected I.V. and the other half I.C. A group of 8 rats was killed at each of the following times after injection: 10 and 30 min and 1, 2, 4, 8, 16 and 24 hr.

RISA

0·1 ml. of a solution of radio-iodinated serum albumin (RISA) (10 μc/0·1 ml.) in isotonic NaCl solution was injected into each of 64 rats. The rats were in groups of 8; one half of each group received the tracer via the external jugular vein and one half via the cisterna magna. A group of 8 rats was killed at each of the following times after injection: 15 and 30 min and 1, 2, 4, 8, 16 and 24 hr.

RESULTS

Iodide

In Fig. 1 the ordinate is radioactivity (counts/min/g wet tissue) plotted semi-logarithmically as a function of time from 30 min to 43 hr after injection of iodide. This method of presentation shortens the ordinate and shows not only the levels of iodide in each tissue at the sampling times represented by the solid and open symbols but also permits interpolation to any time between points. Also, the rate of movement of iodide into or out of each tissue at any time is indicated by the slope of the curve at that time. In Fig. 1, as in those presented subsequently, the symbol

and the bracketed lines represent, in nearly all cases, the mean of 4 or more observations ± S.E.; the curves have been fitted by eye.

A number of interesting features are presented by the iodide distribution curves in Fig. 1. It is apparent that the ^{131}I levels in all the tissues studied were decreasing rapidly by the time the first tissue iodide measurements were made at 30 min. This was true after I.P. (solid curves) and

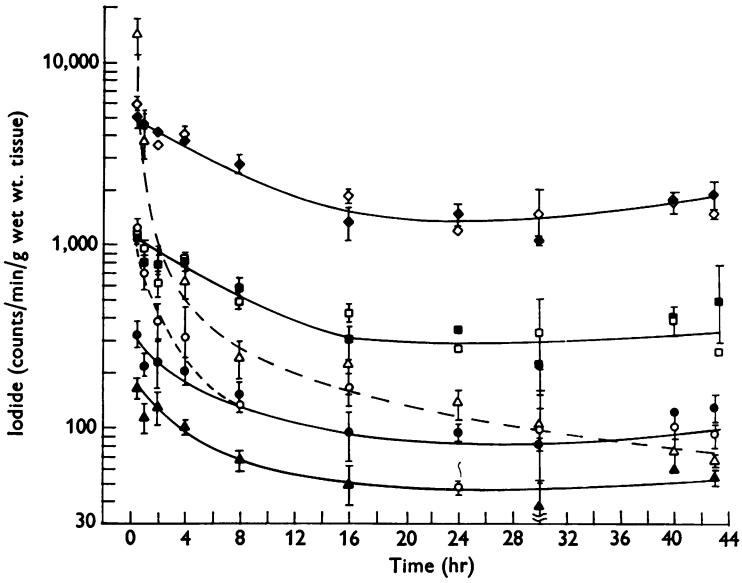

Fig. 1. The radioactivity of various tissues and body fluids of rats expressed as a function of time after I.C. or I.P. injection of $Na^{131}I$ (10 μc/rat) in 0·1 ml. of isotonic NaCl solution. In this and subsequent figures (unless otherwise stated) the following conventions apply: --△--, c.s.f. after I.C. injection; —▲—, c.s.f. after I.P. or I.V. injection; --○--, brain after I.C. injection; —●—, brain after I.P. or I.V. injection; --□--, muscle after I.C. injection; —■—, muscle after I.P. or I.V. injection; --◇--, plasma water after I.C. injection; —◆—, plasma water after I.P. or I.V. injection. Vertical bars represent ± the standard error of the mean. A logarithmic scale is used on the ordinate. The vehicle for the tracer is 0·1 ml. isotonic NaCl solution. All animals had been nephrectomized for 24 hr or for the time from injection to killing, whichever was longer.

I.C. (interrupted curves) injections and it demonstrates that iodide does exchange rapidly between plasma and the various compartments of the C.N.S. and also that it can be rapidly removed from the c.s.f. Wallace & Brodie (1940*a*) observed that iodide passes promptly into c.s.f. after I.V. administration. Of particular interest is the short time required for the c.s.f. iodide values to become less than the plasma iodide after I.C. administration. Within 45 min after injection the c.s.f. iodide concentration was

less than that in the plasma water, and it was one-fourth that of the plasma concentration after 2 hr; the c.s.f.:plasma ratio continued to decrease throughout the remainder of the experiment.

The brain iodide concentration also decreased rapidly after I.C. administration until the brain:plasma iodide ratio after I.C. injection approached the brain:plasma iodide ratio observed following I.P. injection and, after about 8 hr, remained nearly constant for the remainder of the 43-hr observation period.

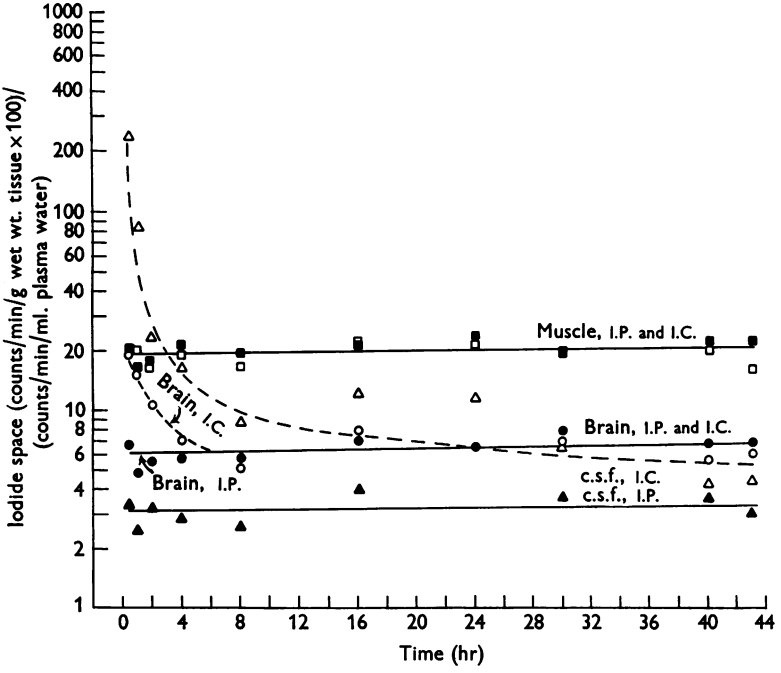

Fig. 2. Iodide space (counts/min/g wet wt. tissue)/(counts/min/ml. plasma water) × 100 of c.s.f. brain, and muscle. The values shown were derived from the data presented in Fig. 1.

In order to visualize more clearly the relation between the tissue iodide and the plasma iodide values, the various iodide spaces (space = (counts/min/g wet tissue × 100)/(counts/min/ml. plasma water)) are shown in Fig. 2. This presentation of the data clearly demonstrates the constant relation between the various tissue and plasma iodide values after I.P. administration. Extrapolation of the curves to zero time shows that the c.s.f. iodide space is about 3 %, the brain iodide space 6 %, and the muscle iodide space 19 %. The term 'space' when used in conjunction with a tracer (e.g. 'c.s.f. iodide space') is defined as outlined above and is used as a con-

venient way of expressing the relation between the tissue and plasma-water tracer activities. It is not intended to connote a discrete anatomical volume. Davson (1955) reported that the c.s.f.:plasma ratios in rabbits varied from 0·04 to 0·004 (c.s.f. iodide space of 0·4–4 %) whereas Wallace

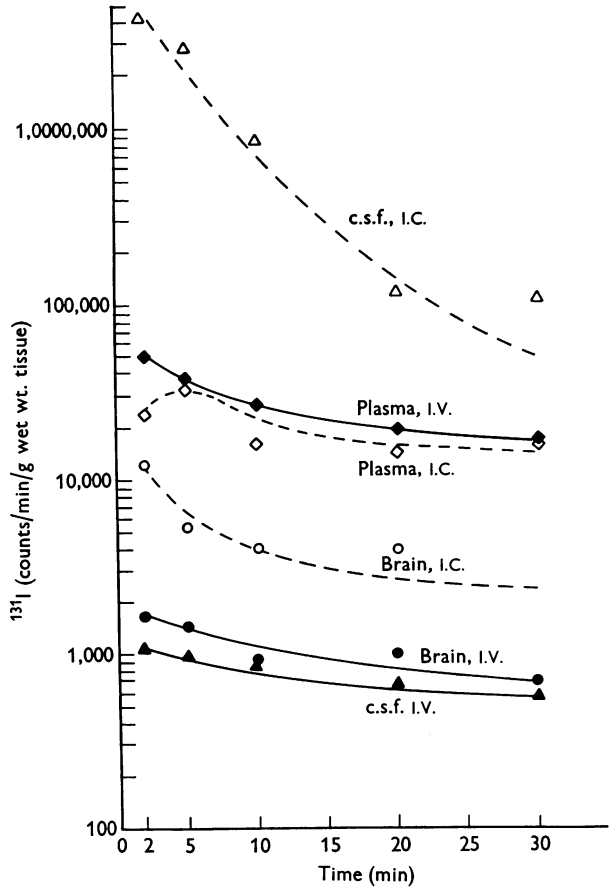

Fig. 3. Radioactivity in tissues of rats from 2 to 30 min after I.V. and I.C. injection of Na^{131}I.

& Brodie (1939) found in dogs c.s.f. iodide spaces of 27–39 % and brain spaces of 8–14 %, after doses of NaI 1–1·5 g/kg. In the dog with constant c.s.f. drainage, Greenberg *et al.* (1943) observed a c.s.f. iodide space of 12 %. Even after I.C. administration, when one might expect the brain and c.s.f. iodide concentrations and spaces to be much larger than after I.P. administration, they rapidly approach the I.P. values.

The fact mentioned earlier that at 30 min all the tissue iodide values were decreasing rapidly suggested the desirability of studying the various

iodide levels during the first 30 min after injection. Thus, the second iodide experiment was performed; the results are shown in Fig. 3. The extremely rapid distribution of iodide is even more apparent in this figure. With the single exception of the plasma iodide after I.C. injection, all the tissue iodide concentrations at 5 min were less than at 2 min after

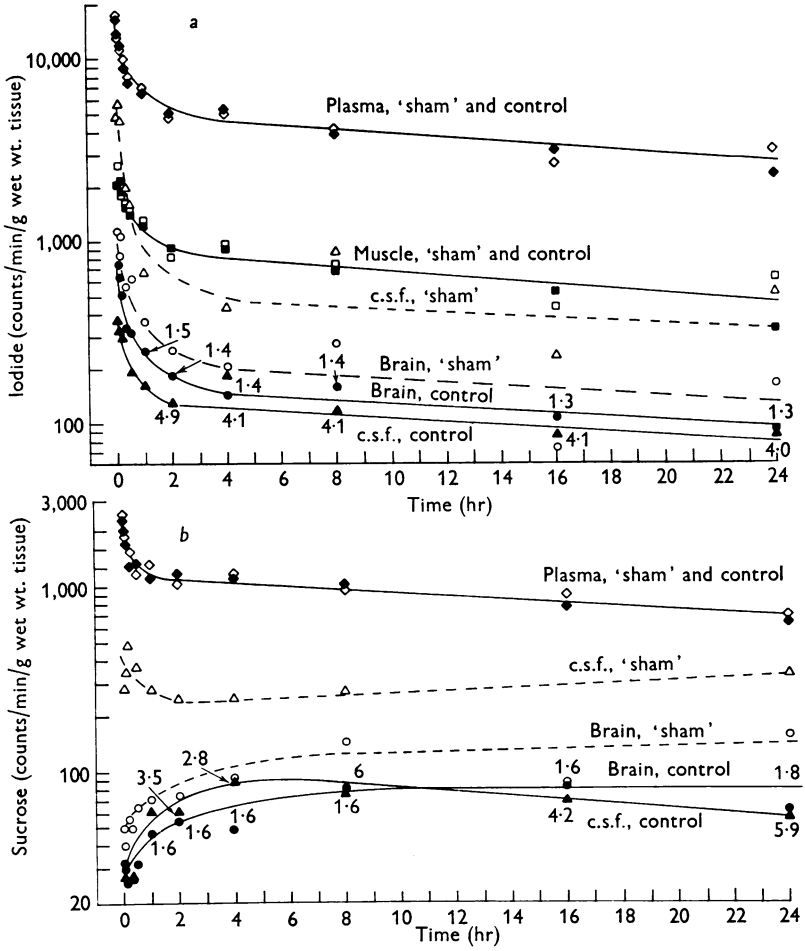

Fig. 4. *a*. The effect of an I.C. injection of isotonic NaCl solution 0·1 ml. on ^{131}I distribution after I.P. injection of 10 μc $Na^{131}I$ is shown by a comparison of the curves labelled 'control' and 'sham'. The 'sham' curves depict the response to the I.C. injection of the saline solution, the 'control' curves represent the response to the I.P. injection only. The numerals associated with the 'control' curves for brain and c.s.f. are the factors by which the corresponding 'sham' curves must be corrected to be made comparable to the 'control' curves. *b*. The description for *a* applies to this figure if ^{14}C-sucrose (4 μc/rat) is substituted for $Na^{131}I$. Both tracers were injected simultaneously.

injection. The plasma iodide after I.C. injection required about 5 min to reach the maximum value.

The experiments in which labelled erythrocytes and plasma proteins were used to measure the volume of residual blood in brain and muscle indicated that $0{\cdot}60 \pm 0{\cdot}008$ % of the brain and $0{\cdot}95 \pm 0{\cdot}034$ % of the muscle volume was blood (after sampling as outlined in Methods).

The results of the attempt to evaluate the effect of an I.C. (sham) injection on the distribution of iodide administered I.P. are shown in Fig. 4*a*. The curves representing iodide activity in the plasma of the sham and control rats are not different, which suggests that any differences between the two groups cannot be attributed to differences in dose. The identity of the two sets of muscle iodide values further indicates that any differences that exist between the two c.s.f. curves and between the two brain curves are associated with the I.C. injection. A comparison of the sham and the control brain-iodide curves and also of the sham and the control c.s.f.-iodide curves suggests that the sham injection *per se* did affect the iodide distribution in the C.N.S. The numbers appearing on the control c.s.f. and brain curves in Fig. 4*a* are the factors by which the sham injection increased the c.s.f. or brain iodide from the control level. The iodide activity in the sham animals is about 4·1 times higher in the c.s.f. and 1·4 times higher in the brain. These relations are quite constant throughout the 24 hr period, with a tendency toward being somewhat higher during the early periods (the first hour).

Sucrose

The results of injection ^{14}C-labelled sucrose are shown in Fig. 5. It was mentioned under Methods that two series of experiments were conducted; in one series ^{14}C-sucrose alone was administered, and in the other ^{131}I was injected with the sucrose. There were 7 time periods which were common to both experiments; since the values obtained at these times were not significantly different, the values of both experiments have been combined for statistical treatment and graphic presentation.

I.P. *injection of sucrose.* The curves derived from the data based on the results of I.P. injection of sucrose (Fig. 5) show a number of interesting aspects of the movement into, and distribution of sucrose in, the C.N.S. The concentration of plasma sucrose gradually declined at a constant rate throughout the experiment. The muscle curve can be seen closely to parallel the plasma curve. The brain and c.s.f. curves are uniquely different from the plasma curve. The brain sucrose levels increased from the first measurement at 30 min until the end of the experiment at 43 hr, in spite of a continuously falling plasma-sucrose concentration. The concentration of sucrose in the c.s.f. appeared to increase more slowly than

that in the brain during the first 1–2 hr, and then remained essentially constant for about 30 hr, after which it began to decline rather rapidly.

I.C. *injection of sucrose.* After the injection the plasma sucrose level increased slowly and reached a maximum at about 8–10 hr, which is also

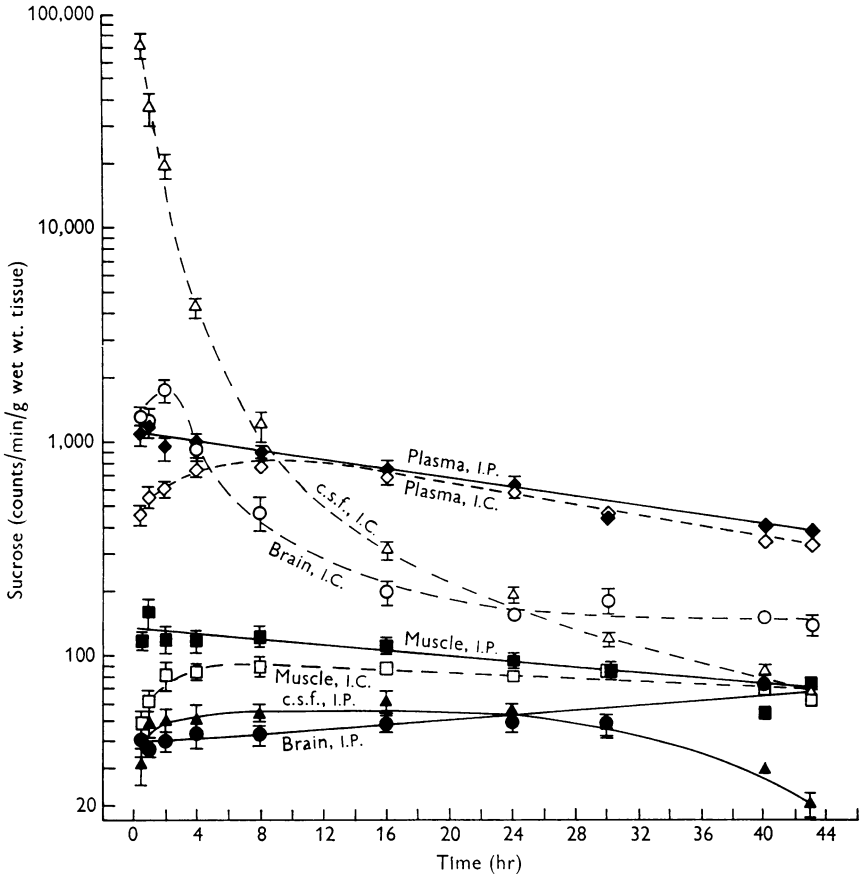

Fig. 5. Tissue radioactivity as a function of time after I.P. and I.C. injection of ^{14}C-sucrose (all values based on a dose of 4 μc/rat). In some animals the dose injected I.P. was twice the activity and volume injected I.C. When corrected to the same dose, the results were not different from those obtained by injecting the same dose and volume by both routes of administration.

the time at which the c.s.f. sucrose level equalled the plasma value. After this time the muscle sucrose curve was nearly parallel to the plasma curve, as after I.P. injection. The brain sucrose concentration was greater than that of the plasma at 30 min, rose during the first 2 hr, was equal to that of the plasma at about 5 hr, and was practically constant after 24 hr. The c.s.f.-sucrose level was very high at 30 min, as might be expected

after I.C. administration, and declined during the first 8–10 hr to a value about 10 % that of the 30 min count and about equal to that of plasma. During the remainder of the experiment the c.s.f. sucrose concentration decreased at a much slower rate; after 26 hr it was less than that of the brain.

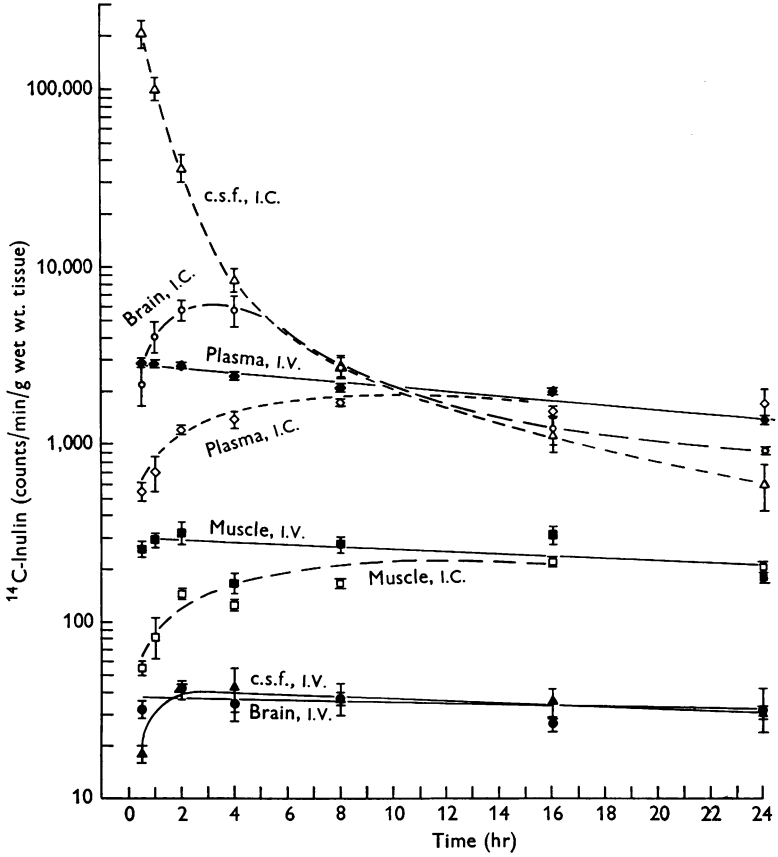

Fig. 6. The ^{14}C activity in brain, muscle, c.s.f., and plasma water after injection of ^{14}C-labelled inulin 2 μc/rat.

In discussing the validity of the data obtained by the methods employed, the possible effects of an I.C. injection on transfer rates and distribution of tracers were considered in some detail. Sucrose and iodide were injected simultaneously in the experiments designed to attempt to quantify the effect of I.C. injection *per se*; the results of the sucrose study are shown in Fig. 4*b*. The sucrose levels were higher in both c.s.f. and brain in the animals which received an I.C. injection of isotonic NaCl solution than in the control animals. The numbers below the control

curves are the factors by which the values obtained from the I.C. injection must be divided to give the same sucrose levels as for the controls. The mean factors are about 4·2 for c.s.f. and 1·6 for brain.

Inulin

The results of I.V. and I.C. administration of inulin are shown in Fig. 6. These results are qualitatively quite similar to those obtained in the study of sucrose distribution, shown in Fig. 5. The only major difference is that between the inulin and sucrose brain curves after I.V. injection. The brain-sucrose curve continued to increase slowly after the first 30 min, while the comparable inulin curve was practically level.

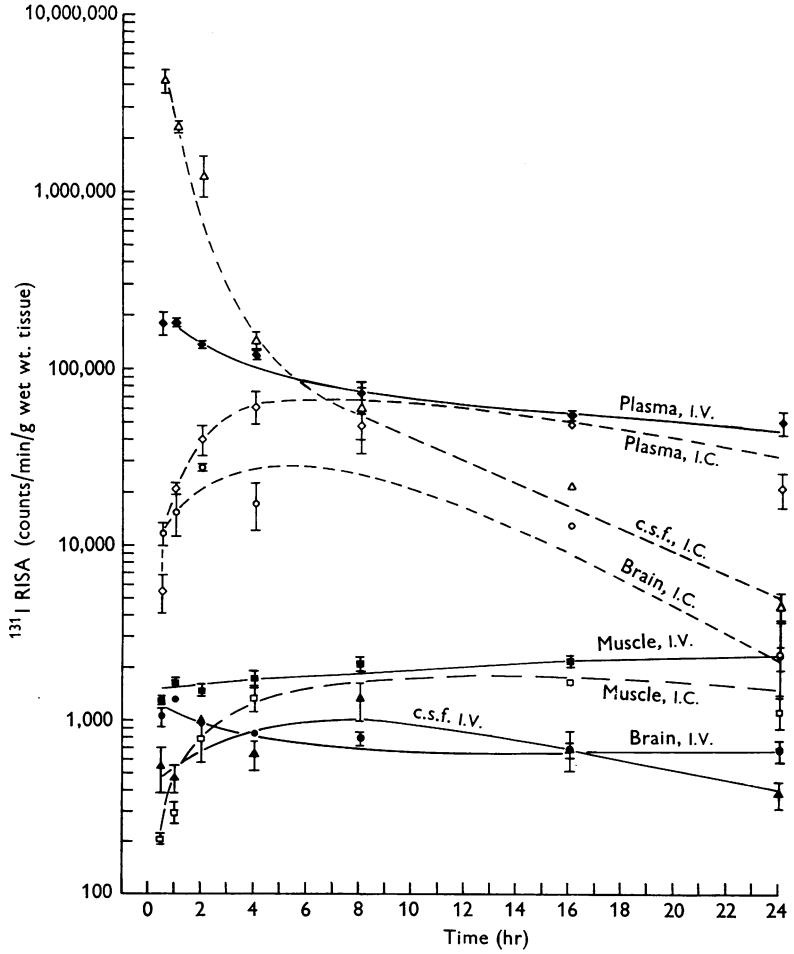

Fig. 7. Radioactivity of brain, muscle, c.s.f., and plasma water from 30 min to 24 hr after injection of RISA.

RISA

The curves representing the results of RISA injection are shown in Fig. 7. There are several interesting differences between the uptake and distribution of RISA and those of inulin or sucrose. After I.V. injection the brain RISA curve was practically parallel to the plasma curve; the muscle RISA concentration continued to increase during the 24 hr. Rothschild, Bauman, Yalow & Berson (1955) studied the RISA levels in muscle for 22 days after I.V. injection and found that the muscle activity increased steadily during the first 5 days and then remained constant. Following I.C. injection the plasma RISA activity was higher than the brain activity during practically all the 24 hr period. The c.s.f. RISA values never declined to the comparable brain level during the 24 hr experiment.

DISCUSSION

Effect of cisternal puncture

The complications inherent in any experiment in which substances are administered I.V. and I.C., and the results compared have been presented at some length in the recent review of Dobbing (1961) and will not be discussed here. A strict adherence to his criteria for a valid I.C.–I.V. comparison would prohibit experiments of this type, since, as he has stated, his criteria are quite incapable of absolutely rigid application. The fact that one cannot perform the perfect experiment is hardly justification for avoiding investigation in this area. However, it is imperative that when an experimental procedure necessarily departs from the ideal the possible effects of such a departure should be kept constantly in mind when data are evaluated and conclusions drawn.

An attempt has been made to evaluate quantitatively some factors in the techniques employed which might influence the results obtained. The residual blood volume of the tissues was determined and corrections, when significant, were made. The effects on tracer movements of I.C. injection of substances in isotonic NaCl solution instead of in c.s.f. were evaluated by comparing the results of the I.C. administration of identical quantities of iodide and sucrose in equal volumes of isotonic NaCl solution and of artificial c.s.f. prepared according to the procedure of Merlis (1940). The results of injecting the tracers in NaCl solution were not different from those obtained by injecting the tracers in artificial c.s.f.

It seemed likely that the trauma of introducing a needle into the c.s.f. space and the removal of a quantity of c.s.f. and its subsequent replacement might affect the movement of the tracer introduced into the c.s.f. It was not technically feasible to perform the ideal investigation of this

effect. In lieu of such an ideal investigation, a study was made of the effect of a sham I.C. injection on the rate of entry and distribution of a tracer in the C.N.S. after I.P. injection. It was felt that such a study, when

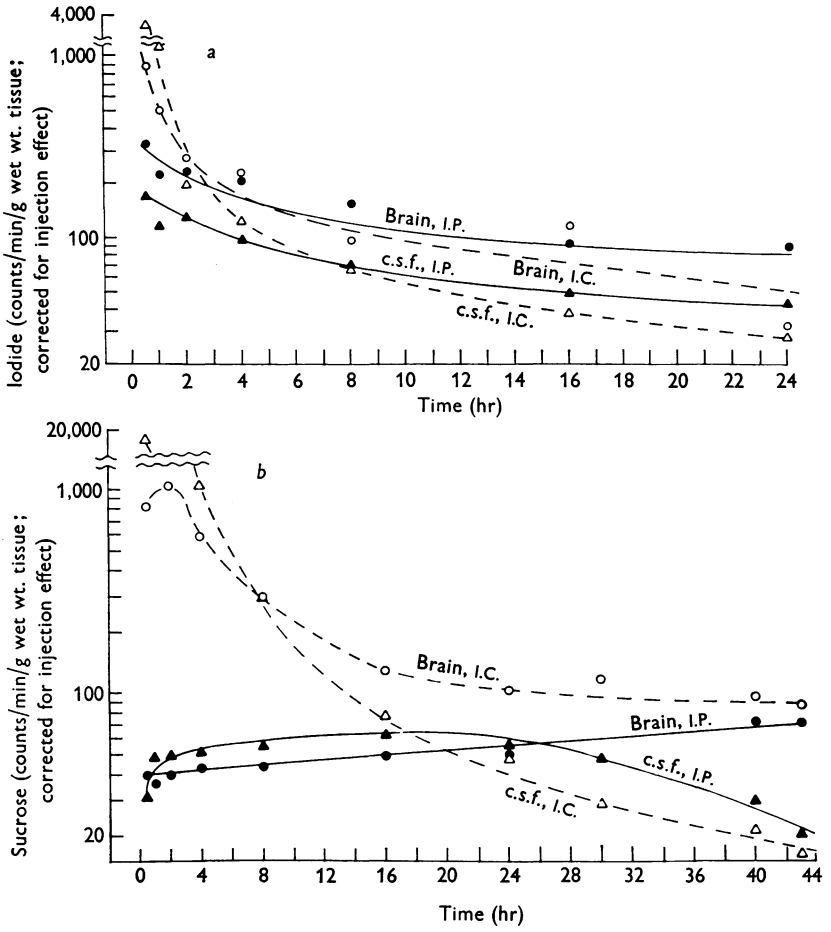

Fig. 8. *a*. The I.P. c.s.f. and brain curves of Fig. 1 are reproduced in this figure (solid curves) and the I.C. c.s.f. and brain curves (interrupted curves) are obtained by correcting the I.C. c.s.f. and brain curves of Fig. 1 by the factors of 4·1 and 1·4, as obtained from the data presented in Fig. 4*a*. *b*. The I.P. c.s.f. and brain curves of Fig. 5 are reproduced here (solid curves) and the I.C. c.s.f. and brain curves (interrupted curves) were obtained by correcting the I.C. c.s.f. and brain curves of Fig. 5 by the factors of 4·2 and 1·6, obtained from the data presented in Fig. 4*b*. Note different abscissa scales.

carried out with multiple tracers, might provide important information about the possible magnitude of the effect of an I.C. injection.

As is shown by the curves of Fig. 4, the c.s.f. and brain tracer activities

after an I.C. injection of saline solution were higher than those for the comparable controls. This may be due to some extravasation of plasma or blood from the injection site; or the trauma incident to the withdrawal of c.s.f. and the injection of saline solution may have affected the integrity or the permeability of the ependyma; the data are not adequate to choose between the two alternatives. A comparison of the curves in Fig. 8 (obtained by applying the factors relating the sham curves to the control curves in Fig. 4*a*, *b* to the brain and c.s.f. curves of Figs. 1 and 5) with the curves in Figs. 1 and 5 shows that the sham injection did produce some quantitative changes in tracer levels but qualitatively the relations between the various tissue tracer levels are not changed. That the possible error as a result of the I.C. injections is much smaller than that suggested by the factors obtained in Fig. 4 is shown by the fact that most of the corrected (I.C.) curves are now lower than the I.P. curves (Fig. 8). It is worthy of note that although iodide and sucrose are quite different with respect to rates of movement between compartments as well as to their concentrations in those compartments in the C.N.S., the correction factors for the sham injection of saline solution following I.P. administration of the two tracers are almost identical. Since the magnitude of the I.C. injection effect in the experimental situation must have been much less than the values derived from the sham experiments and since the qualitative relations were not altered, the data as presented have not been corrected for the effect of the I.C. injection.

Iodide

The uptake and distribution of iodide (as well as the other tracers) in muscle were studied, and the data are included in this report for two important reasons. First, it is necessary to compare the results in tissues of the C.N.S. with non-neural tissue in order to appreciate the difference in the way iodide is handled within the C.N.S.; muscle serves as a good tissue for comparison. Secondly, since muscle has been rather widely studied, the data obtained for muscle serve as an internal control for the experiment. Thus, if the values for muscle agree closely with those previously obtained for this tissue, one can assume that the technical aspects of the experiment and the analyses are valid.

In view of the comparisons to be made it should be pointed out that the muscle-space curve (see Fig. 2) is essentially a horizontal line with a zero intercept of about 19 %. This means that iodide in the muscle maintained a constant relation to the plasma iodide. This figure for muscle iodide space is in good agreement with values obtained by Halmi, Stuelke & Schnell, (1956) for the rat (18 %), and Wallace & Brodie (1937) for the dog (17 %).

Whether the 5–6 % iodide space in the brain bears the same relation to the brain extracellular space as the 19 % iodide space in muscle does to the muscle extracellular space (chloride space = 14 %) is not known. To correlate the brain iodide space with the brain extracellular space it is necessary to decide whether the iodide of the brain is in equilibrium with the iodide of the plasma or with that of the c.s.f. Wallace & Brodie (1939) interpreted their data as showing that the E.C.F. of the brain was in equilibrium with the c.s.f., not with the plasma. In contrast, the data presented in Fig. 1 support the view that the factor which determines the brain, c.s.f., and muscle-iodide levels is the plasma-iodide concentration. After I.P. injection of iodide, the muscle, brain, and c.s.f.-iodide curves are essentially parallel to the plasma-iodide curves. Following I.C. administration of iodide the c.s.f. iodide concentration is initially high, as is also the brain-iodide concentration, even though the plasma iodide is not significantly different from the I.P. plasma values. Thus iodide must have moved from the c.s.f. into the brain tissue. However, the c.s.f. iodide concentration falls below the brain-iodide level even after I.C. injection. The magnitude of the fall in c.s.f. iodide concentration is adequate to reverse the existing c.s.f. to brain iodide concentration gradient. This is true whether all the iodide in the brain is uniformly distributed in brain water or is limited to an extracellular space as small as 6 % (iodide space). Thus, the iodide concentration in c.s.f. water becomes less than that in brain water or brain E.C.F. If the brain iodide is in dynamic equilibrium with the c.s.f. rather than with the plasma, iodide must be actively transported from c.s.f. to brain. Since the c.s.f. iodide declines until its relation to the plasma iodide is essentially the same as after I.P. administration, it seems more likely that, although iodide can and does move between c.s.f. and brain, the plasma iodide level is the primary determinant of the equilibrium c.s.f. and brain levels even after I.C. injection, except for the few minutes immediately following such injection.

The equilibrium c.s.f.:plasma iodide ratio of about 0·03 would indicate that iodide is either (1) excluded from the c.s.f. except for possibly a small 'leak', or (2) actively removed from the c.s.f., or (3) handled by both methods. Since the removal of substances from the c.s.f. by the flow of c.s.f. into the blood via the arachnoid villi, as indicated by the results with sucrose, inulin, and RISA, is completely inadequate to explain the rapid fall in c.s.f. iodide levels after I.C. injection, and since the c.s.f. iodide levels are lower than those in the plasma or the brain, it seems apparent that iodide must be actively transported out of the c.s.f. Welch (1962) has shown that *in vitro* the rabbit choroid plexus concentrates iodide from the bathing medium as much as 30 times. The choroid plexus may contribute to the maintenance of the c.s.f.:plasma iodide ratio by

pumping iodide out of the c.s.f. or by actively preventing its entry. The choroid plexus would seem unable to maintain a c.s.f.:plasma iodide ratio of 0·03 unless iodide does not penetrate the ependyma or the ependyma actively participates in the exclusion or removal of iodide from the c.s.f. The fact that the brain iodide level is much higher after I.C. than after I.P. injection when the plasma levels are equal indicates that iodide transfers passively or actively from the c.s.f. to brain without first entering the plasma. That the c.s.f. iodide level after I.C. administration becomes less than the brain level would suggest that the participation of the ependyma is active. There is an alternative explanation of these findings, however, which does not require the active extrusion of iodide by the ependyma. We suggested (Reed & Woodbury, 1960), on the basis of blood–brain barrier studies with sucrose, the possible existence of a bulk flow of fluid from the plasma into the c.s.f., from the c.s.f. into the brain tissue, and from the brain tissue back into the plasma. This is in addition to the commonly accepted flow of c.s.f. into the plasma via the arachnoid villi. The operation of such a system is discussed later when the data for the various tracers are compared.

Sucrose

The plasma curves of Fig. 5 indicate that after I.C. administration sucrose leaves the c.s.f. and enters the blood stream rather slowly, since 8–16 hr was required for the plasma levels (after I.P. and I.C. injection) to become identical. The steady decline in the plasma sucrose levels after the maximum value was reached is probably due primarily to diffusion into slowly equilibrating tissues.

After I.P. injection the constant slow rise in brain sucrose levels for 43 hr, preceded by a rapid rise, when considered in association with the falling plasma level, suggests that sucrose distributes in at least two compartments at quite different rates. The 'fast' compartment may represent the entry of sucrose into extracellular space and the 'slow' compartment may represent entry into cells. We have considered the possibility that the slow uptake of sucrose in brain may be associated with its entry into glial cells, and this problem is currently under investigation. To illustrate more clearly the change in the sucrose space of brain and c.s.f., the sucrose space of each is plotted in Fig. 9. An extrapolation of the I.P. brain sucrose-space curve to zero time shows the fast compartment to be about 3–4 %. The size of the slow compartment cannot be ascertained from these data, since the space is still increasing at 43 hr. However, the difference between the I.P. 43 hr sucrose space (19 %) and the fast compartment (4 %) and the difference between the I.C. 43 hr sucrose space (42 %) and

the fast compartment (4 %) probably delineate the limits (15–38 %) of the size of the slowly equilibrating sucrose space of the brain. The slow prolonged increase in brain sucrose space is in marked contrast to the practically constant brain inulin space and brain iodide space.

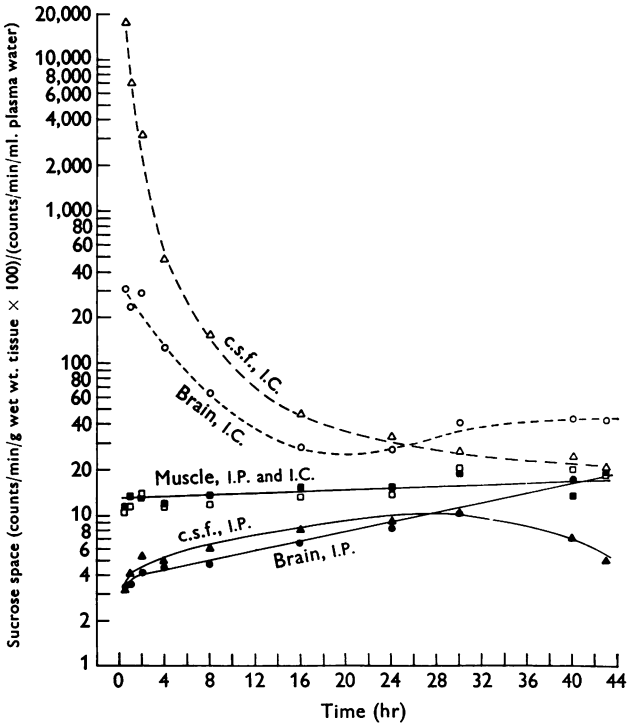

Fig. 9. The sucrose spaces of muscle, brain, and c.s.f. after I.P. and I.C. administration as a function of time after injection.

Inulin

In many respects the results obtained with inulin (Fig. 6) are quite similar to those discussed above for sucrose. After I.V. injection the plasma inulin levels declined more slowly than did the plasma sucrose. This may be due to a difference in permeability and/or volume of distribution. The brain inulin concentration is practically constant. Following I.C. administration of inulin the plasma inulin levels increased slowly and required about the same time as did the plasma sucrose values to reach a maximum (8–12 hr). Of interest is the longer time, compared to sucrose, for the brain value to reach its maximum. These differences indicate that inulin moved from c.s.f. to plasma and from c.s.f. to brain somewhat more

slowly than sucrose. The significance of this difference will be discussed in detail in the general discussion to follow.

The inulin space provides a good indicator of the relation between the various tissue inulin concentrations and the plasma inulin values. The inulin spaces are shown in Fig. 10. The muscle inulin space is about 12 % after either route of administration, and is nearly constant for 24 hr.

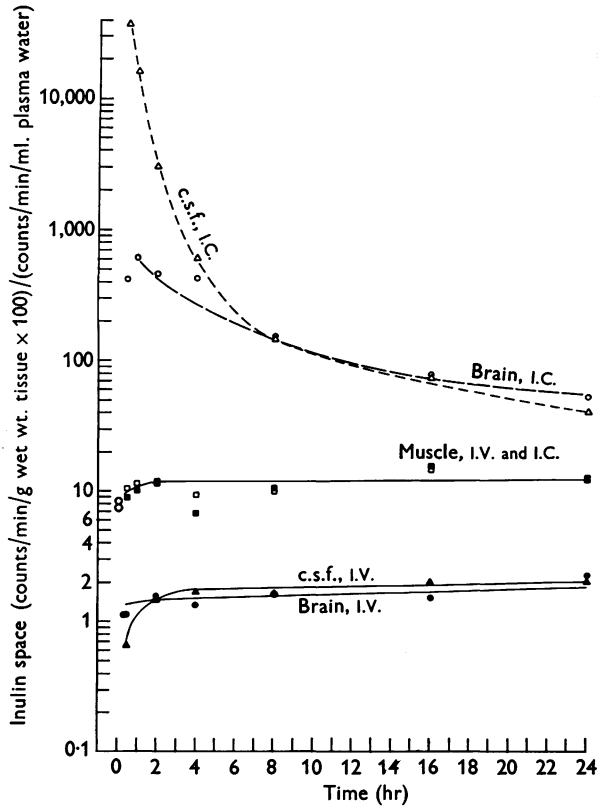

Fig. 10. The inulin spaces of muscle, brain, and c.s.f. after I.V. and I.C. administration as a function of time after injection.

Conway & Fitzgerald (1942) reported the inulin space of rabbit muscle was 8·5 % in *non-nephrectomized* animals. The muscle inulin space agrees closely with the muscle chloride space (14 %) and the muscle sucrose space (14 %), and this agreement supports the concept of the extracellular distribution of inulin and sucrose in muscle. After I.V. injection the brain and c.s.f. inulin spaces are small, 1·5–2 % These values agree well with the inulin spaces reported by Morrison (1959), as follows: brain, 2 % for dogs, 3 % for rats; and c.s.f. 2–3 % for dogs As with muscle, these spaces are practically constant throughout the experiment. The brain- and

muscle-space curves support the concept that inulin is extracellularly distributed.

RISA

After I.V. injection the decrease in brain radioactivity was parallel to the decrease in plasma radioactivity, while the muscle albumin levels continued to increase (Fig. 11). This is the converse of the results obtained

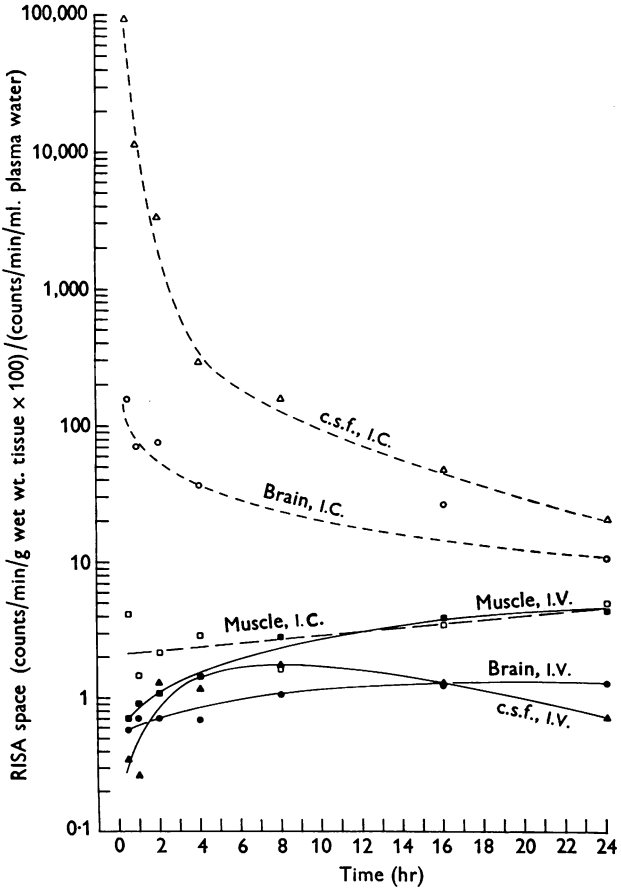

Fig. 11. RISA spaces of muscle, brain, and c.s.f. from 30 min to 24 hr after I.V. and I.C. injection of RISA.

with sucrose, and suggests that albumin left the vascular compartment and slowly entered the extracellular space of muscle. However, the results for brain suggest that albumin did not leave the vascular bed. The plot of RISA tissue spaces (Fig. 11) shows that the brain RISA space was only

about 1%. Since the residual plasma in the brain was measured and found to be 0·60%, and allowing for a small amount of free iodide in the RISA (less than 1%), all the brain iodide can be accounted for without albumin having entered the brain tissue. Similarly, the c.s.f.-space curve suggests that little if any albumin entered the c.s.f. after I.V. administration. Rozdilsky & Olszewski (1957) reported that RISA did not cross the blood–brain barrier, and Brown (1961) reported only small amounts of radioactivity in brain and c.s.f. after I.V. injection of RISA. Other investigators have also concluded that RISA does not enter the c.s.f. after I.V. administration. Wasserman & Mayerson (1951) found no radioactivity in the c.s.f. for periods up to 5 days after injection of varying doses of RISA. Sweet, Brownell, Scholl, Bowsher, Benda & Stickley (1954) detected minute amounts of radioactivity in the c.s.f. of some patients after RISA administration. The findings of Fishman (1953) are in sharp contrast to those cited above and also to the data presented in this paper. He reported radioactivity in the c.s.f. as early as 40 min after injection of extremely high doses of RISA (1–4 mc/dog) with equilibrium values (i.e. same specific activity as plasma) at 17–25 hr.

That albumin can enter the brain tissue from the c.s.f. is indicated by the large RISA brain space (10–150%) after I.C. injection. The fact that the brain RISA levels decrease rather rapidly after the maximum is reached at 5–7 hr (Fig. 7) and are always less than the plasma values after the first 30 min raises the interesting question as to how RISA is removed from the brain tissue. It is conceivable that RISA moves directly from the brain into the plasma, although the I.V. experiments show that it does not move in the opposite direction. However, if RISA is confined to an extracellular brain space of about 5–6%, the diffusion gradient would be from brain to plasma. Similarly after 6 hr (at the time when the c.s.f. concentration becomes less than the plasma level, Fig. 7), there would be an even greater gradient between brain E.C.F. and c.s.f. than between brain E.C.F. and plasma. Since the albumin entered the brain from the c.s.f. and since the I.V. experiments showed that albumin did not cross the blood–brain interface, it seems more likely that RISA leaves the brain by re-entry into the c.s.f. and thus into the blood via the arachnoid villi. Lee & Olszewski (1960), by use of an autoradiographic technique, showed that RISA enters the brain from the c.s.f. after intrathecal administration. They also concluded that RISA probably left the brain by re-entry into the c.s.f. Although it has been shown that certain substances can move from the c.s.f. into the brain and then into the plasma, some materials are excluded from this pathway. RISA appears to be among those excluded, and therefore may be a good indicator of c.s.f. flow through the arachnoid villi into the plasma.

Evaluation of combined findings from all tracers

The references cited in the introduction clearly indicate that the use of iodide, sucrose, inulin, and RISA for investigating the blood–brain barrier and measurement of tissue spaces is not new. However, the use of these substances and the application of our techniques to a large, homogeneous population of animals and the obtaining of simultaneous brain, c.s.f., muscle, and plasma tracer activities at many times during an extended period seem to be unique. The significance of any rate or concentration measured in a single compartment is greatly enhanced by a knowledge of the simultaneous rates and concentrations in the adjacent compartments.

Perhaps the most productive approach to evaluation of the data presented is by comparing the results obtained with the four tracers for each parameter studied (c.s.f. activity, brain activity, spaces, etc.). Thus the significance of a change in concentration or rate of movement of an isotope may be more readily understood and correctly interpreted if viewed in the light of changes observed with other isotopes under the same conditions.

After I.P. or I.V. injection, the plasma is the source of the tracer which enters the C.N.S. The plasma levels produced by injection of a standard amount of activity will depend on the rate of exit from the vascular bed and the volume of distribution of the tracer in all body tissues. Thus the plasma levels of the four isotopes studied, if identical amounts of each are injected simultaneously in the same animal, would be different at any measurable subsequent time. This source of variation can be circumvented by comparing the tissue:plasma ratios or the tracer spaces. Such a plot of the brain tracer spaces as determined by the different tracers is shown in Fig. 12. The brain tracer space following I.P. or I.V. injection appears to be inversely related to the molecular weight of the tracer substance. However, as was pointed out in the discussion of RISA, the brain RISA-space curve probably represents the activity associated with the residual blood and slight contamination of the albumin with free iodide. It seems probable that albumin did not enter the brain from the plasma. The brain tracer spaces obtained by extrapolation to zero time are as follows: inulin, 1·5–2·0%; sucrose, about 4%; and iodide, 6%. The space curves obtained from I.C. injection of the tracers are also shown in Fig. 12. It is essential that several facts be kept in mind in interpreting the significance of the brain-space curves under these conditions. After I.P. or I.V. administration, when for all practical purposes the isotopes were placed directly in the vascular compartment, the plasma levels were initially high and quite stable, and hence the space curves are a reflexion of the rate at which the tracers entered the brain. However, as demonstrated by this study, after I.C. administration the plasma is not initially

the primary source of the brain tracer, and the plasma levels are dependent upon the rate of exit of the substance from the c.s.f. and possibly from the brain. Also the brain levels are affected not only by the rate of entry from the c.s.f. but also by exchange with the plasma. Thus the brain space for a material which enters the brain rapidly from the c.s.f. may be much

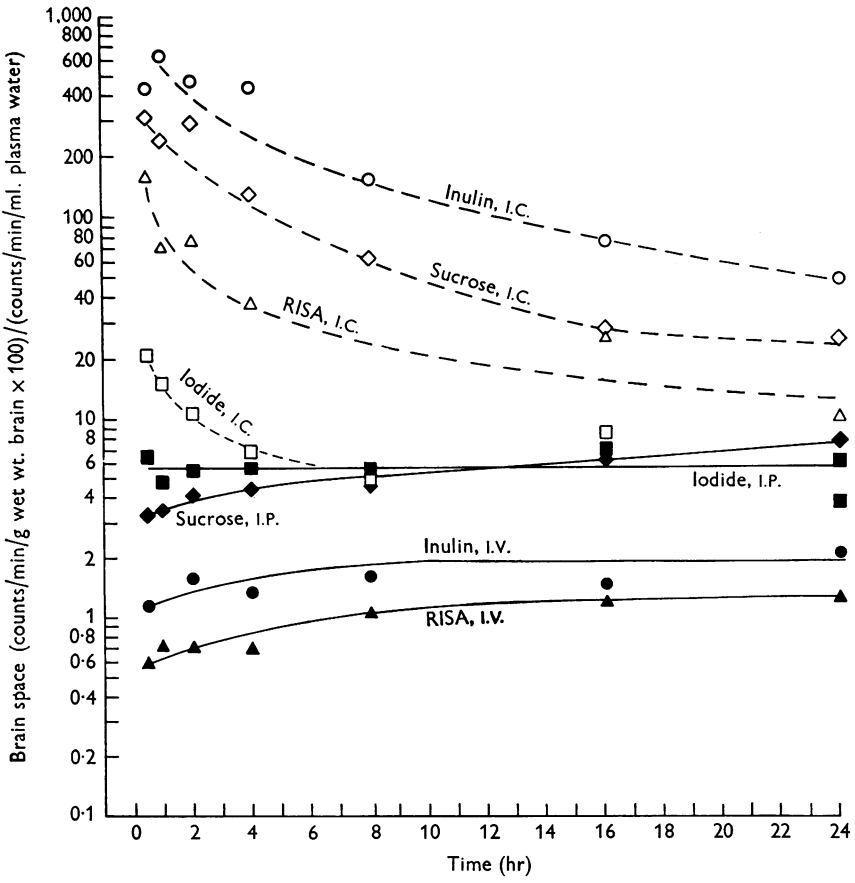

Fig. 12. The brain space, as measured by each of the four tracers used, plotted on the same co-ordinates: ○, inulin space; ◇, sucrose space; □, iodide space; △, RISA space. Open symbols for I.C. injection of the tracers, solid symbols for I.P. or I.V. administration.

smaller than the comparable space for a slowly exchanging substance if the former can also exchange rapidly between the brain and plasma. This probably explains why 30 min after I.C. injection the iodide space is much less than the RISA space and also much less than the sucrose and inulin spaces. On this basis one might predict that, after I.C. injection, the brain

tracer space at any time as measured by the various isotopes would increase in this order: iodide, sucrose, inulin, and RISA. But, as can be seen from the I.C. curves in Fig. 12, the RISA curve does not fit into this predicted sequence. It was shown that RISA does not enter the brain from the plasma to a measurable degree and it seems logical that it probably does not move in the opposite direction. Also it will be shown that RISA movement from c.s.f. to brain is much slower than for the other tracers. Thus, if the rate at which RISA enters the brain as compared to its entry into the blood was much slower than the comparable rates of entry of sucrose and inulin, the brain RISA space would be expected to be lower than the sucrose and inulin spaces, as was observed. However, as pointed out above, the rate of exchange between brain and plasma will also affect the brain spaces.

This raises the interesting question as to the relation between the various brain tracer spaces presented above and the true extracellular space of the brain. This question will be considered later in conjunction with the discussion of possible mechanisms which might account for the observed distribution of substances in the C.N.S.

The c.s.f. tracer spaces after I.V. or I.P. administration are shown in Fig. 13. The c.s.f. RISA (I.V.) space curve suggests that a minute amount of albumin may have entered the c.s.f. from the plasma. Also the c.s.f. space seems to be inversely related to the molecular weight of the tracer, as was the case in the brain, except that the iodide spaces are smaller than the comparable sucrose spaces. Iodide entry into the c.s.f. from the plasma is much more rapid than for the other substances, as shown by the fact that at 30 min the iodide space is already maximum.

Several features pertaining to the c.s.f. space curves after I.C. injection (upper curves, Fig. 13) are of interest relative to the movement of substances out of the c.s.f. First, the iodide-space curve differs markedly from the three other curves. This fact, in conjunction with data presented above, indicates either that there are pathways available for iodide exit from the c.s.f. which are not available to the other tracers, or that, if the same pathways are available for all, some are of much greater importance for iodide movement than for sucrose, inulin, and RISA movement. Secondly, the rate at which the tracers move out of the c.s.f. during the first 30 min after injection is markedly different, as shown by the difference between the 30 min c.s.f.-space values (initial points, Fig. 13). The c.s.f. RISA space is about 400, 5 and 2·5 times the iodide, sucrose and inulin spaces, respectively, at this time. If the rate at which various substances leave the c.s.f. via the arachnoid villi is mainly independent of molecular size (Prockop & Schanker, 1962), then the variation in 30 min spaces noted above is probably due to differences in (*a*) the rate at which the

tracers enter the brain tissue, and (*b*) the rate at which they leave the plasma and enter other tissues. Thirdly, after about 2 hr the sucrose-, inulin-, and RISA-space curves are practically the same. This may be fortuitous. However, it is probably associated with the fact that by 2–4 hr

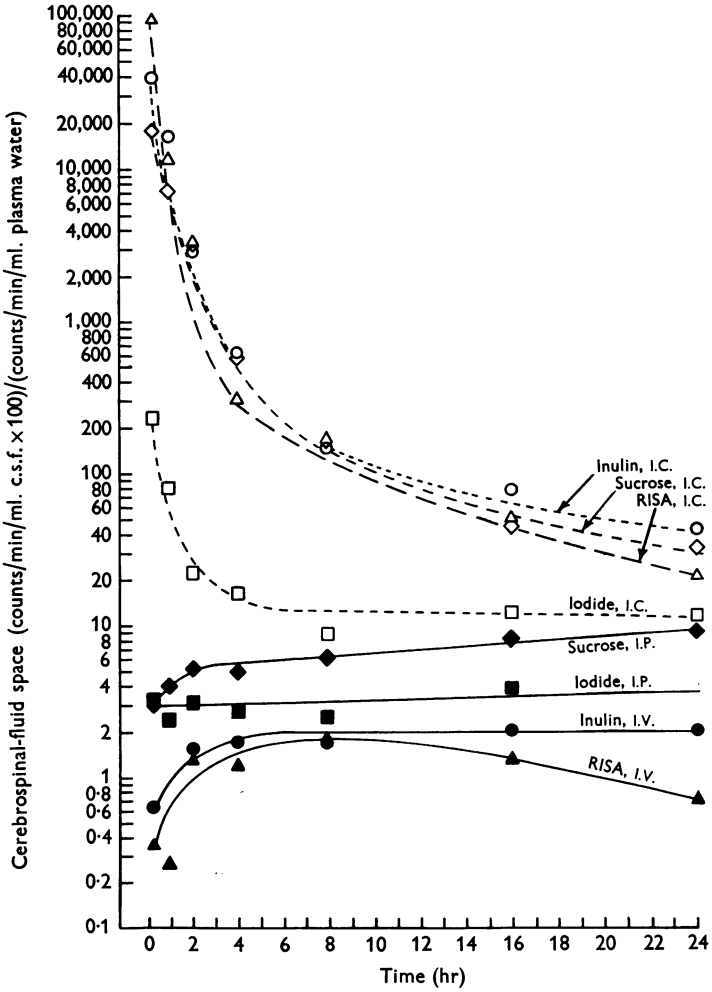

Fig. 13. The c.s.f. space curves obtained with each tracer on same co-ordinates. The legend is the same as that for Fig. 12.

after injection the c.s.f. flow through the arachnoid villi, movement into brain tissue, and mixing in the c.s.f. may have reduced the c.s.f. concentration by as much as 90–95 % of the injected activity. This may explain why Prockop & Schanker (1962) found that the amounts of tracers remaining in the c.s.f. 6 hr after intraventricular injection of inulin,

sucrose, and mannitol were similar. Thus the other avenues of egress which significantly influenced the early c.s.f. levels become relatively unimportant as factors affecting c.s.f. values at this later time. This interpretation also emphasizes the fact that the movement of iodide out

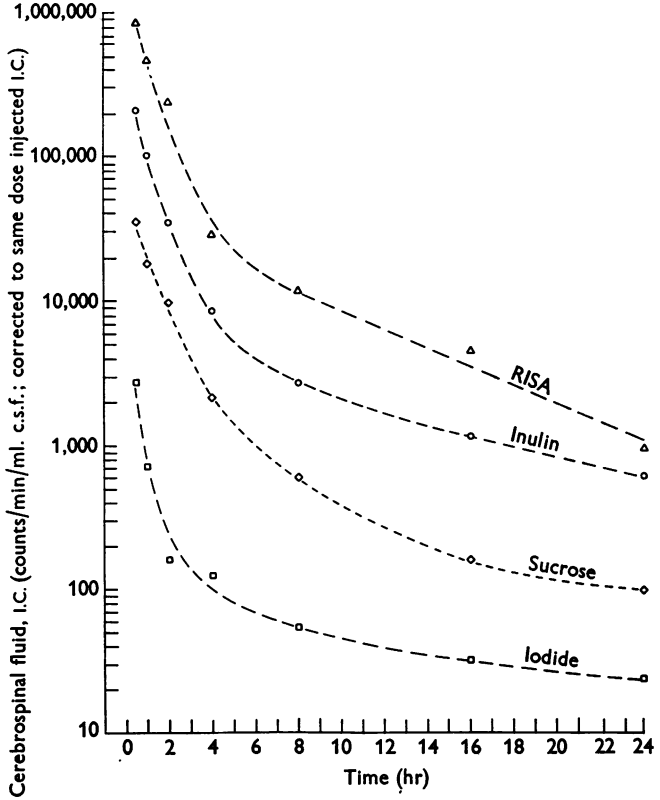

Fig. 14. The c.s.f. curves were obtained by correcting the I.C. c.s.f. curves obtained with each tracer to the same injected dose: they are plotted as a function of time from 30 min to 24 hr. △, results obtained with RISA; ○, inulin; ◇, sucrose; □, iodide.

of the c.s.f. by means other than flow through the arachnoid villi must be of major import.

Although the similarity of the sucrose-, inulin- and RISA-space curves suggests that a common mechanism probably accounts for most of their movement out of the c.s.f. compartment, it tends to obscure the relative importance or even the existence of other mechanisms. The 30 min c.s.f. space values and also the brain space values after I.C. injection suggest that there are other means of tracer movement out of the c.s.f. in addition to flow through the arachnoid villi. If such avenues do exist then a plot

of c.s.f. activity for each tracer as a function of time after injection of the same amount of activity should give an estimate of their relative importance. Such a plot of the counts/min/ml. c.s.f. corrected to the same injected dose is shown in Fig. 14. It is apparent that, at any time during the experiment, the RISA activity is greater than inulin > sucrose > iodide. Thus it appears that either there is selective passage through the arachnoid villi for the soluble substances studied or there are pathways

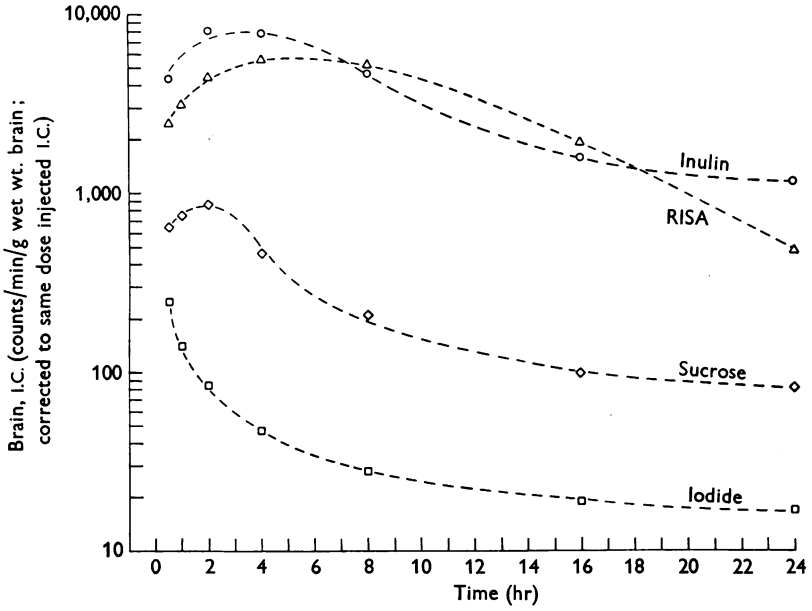

Fig. 15. I.C. brain curves obtained in the same manner as were the c.s.f. curves of Fig. 14. The legend is the same as for Fig. 14.

available for the movement of inulin, sucrose, and iodide which are not equally available to RISA.

That this additional loss of tracers from the c.s.f. involves entry into or passage through the brain is shown rather conclusively by the curves in Fig. 12, in which the brain spaces after I.C. injection are many times higher than the comparable spaces after I.P. administration. The plot of brain activity after correction to the same injected dose of each tracer (Fig. 15) not only shows that appreciable amounts of activity are in the brain but also indicates the rate of entry into this tissue. The rate of entry is related to the time required for the maximum brain levels to be attained. The approximate times to attain the maximum levels of each isotope were as follows: iodide, less than 30 min; sucrose, 2 hr; inulin, 4 hr; and RISA, 6 hr. The absolute level of activity of a tracer is not

necessarily related directly to the rate of entry, since the brain level is the resultant of the rates of *entry* into and *exit* from the brain.

The experimental results suggest that the blood-brain-c.s.f. system has the following characteristics: (1) iodide, sucrose and inulin enter the brain from the blood and also from the c.s.f. (at least under conditions of a high c.s.f. concentration as after I.C. injection), and RISA is excluded from the brain after I.V. but not I.C. injection. The brain:plasma ratio after I.P. or I.V. injection is unique for each substance and during the first 12 hr the range varies from 0·0 (RISA) to 0·06 (iodide). (2) All the tracers studied with the possible exception of RISA enter the c.s.f. directly from the plasma and/or the brain. None of the c.s.f.:plasma ratios is the same and the range varies from a fraction of 1 % to about 9 % during the 24 hr after injection. (3) After I.C. injection all the tracers examined enter the brain from the c.s.f., each at its characteristic rate. Most of the curves representing the decline in activity of each tracer in the c.s.f. after I.C. injection have one component in common, which indicates the presence of a non-selective pathway available to all tracers. It is suggested that this might be flow through the arachnoid villi. If RISA loss is limited to flow through the arachnoid villi, then sucrose, inulin, and iodide have significant additional avenues of escape from the c.s.f., compared to RISA. In fact only a small fraction of the movement of iodide from the c.s.f. appears to be associated with c.s.f. flow through the arachnoid villi. (4) Some transport mechanism must exist to remove iodide from the c.s.f. That this must be so is indicated by the observation that, even after I.C. injection, the c.s.f. iodide concentration falls more rapidly than either the brain or the plasma concentration, and the rate of this fall cannot be accounted for by exit via the arachnoid villi. Although not as clear, the evidence also indicates that sucrose and inulin may be similarly transported.

In view of the observations summarized above, what are the characteristics of the system under investigation which will explain them? Pappenheimer, Heisey & Jordan (1961) have shown that diodrast and phenolsulphonthalein (PSP) are actively transported out of the c.s.f. Our data are compatible with the existence of a pump for iodide and possibly for sucrose and inulin. Although the existence of transfer maxima and responsiveness to inhibitors of active iodide transport have not been demonstrated, they are currently under investigation. That iodide is actively transported is suggested by the fact that the decrease in c.s.f. iodide level is much too rapid to be accomplished by bulk flow through the arachnoid villi, and passive diffusion would appear to be an inadequate explanation, since the rapid decline in c.s.f. iodide concentration continues after the c.s.f. iodide activity falls below that in the plasma or the brain.

Does this mean that a specific pump exists for each substance which has been shown to be actively transported out of the c.s.f. or is there a non-selective transport system which will extrude a range of substances such as iodide, diodrast, PSP, possibly sucrose, and inulin, and probably others? A large number of molecules and ions have been shown to be actively transported by the kidney, and possibly similar conditions prevail in the C.N.S. If such a situation does exist the data presented in this communication would indicate that the transport of substances from the c.s.f. is into the brain as well as into the blood.

In quest of a simpler mechanism to explain the observations reported, we have considered a theoretical system of a type which may best be described by reference to Fig. 16, which shows a highly schematic representation of the C.N.S. The fluid flow and solute movement which are necessary to describe this system are indicated by the arrows. The *solid arrows* depict only fluid flow from blood to c.s.f. and then to brain or arachnoid villi. The *open arrows* depict the diffusion of solutes across the various interfaces. From the blood fluid passes into the c.s.f.; from the c.s.f. a portion of the fluid flows through the brain tissue and back into the blood stream, while the balance re-enters the vascular system via the arachnoid villi. Solutes may enter the c.s.f. from the blood or from the brain tissue. In the case of substances with a low c.s.f.:plasma ratio, direct movement from the blood into the c.s.f. must be greatly restricted or non-existent. If it is non-existent, the source of the solute in the c.s.f. is the brain E.C.F. via diffusion against the current of fluid flow. Thus a low c.s.f.:plasma ratio would be due to restricted solute movement between plasma and c.s.f. by a selectively impermeable membrane and reduced diffusion from the brain tissue into the c.s.f. as a result of fluid flow from the c.s.f. into the brain. Larger c.s.f.:plasma ratios would be dependent upon an increased permeability of the membrane between plasma and c.s.f.

It is believed that the existence of such a system with the proper permeability characteristics at the blood–brain, brain–c.s.f., and blood–c.s.f. interfaces could explain all the observations reported above, relative to the movements and distribution of iodide, sucrose, inulin and RISA, without the necessity of a specific pump for iodide or the other tracers studied. By way of example, consider the results of I.P. or I.C. iodide administration. It was found that the c.s.f. concentration after I.P. or I.V. injection is only about 3% of that in the plasma. Thus the fluid flowing from blood to c.s.f. would be essentially free of iodide, as also would be the fluid flowing from the c.s.f. into the brain tissue. The rapidity with which iodide enters the brain tissue would require a prompt movement of iodide from the blood into the brain E.C.F. The iodide movement

after I.C. injection shows a rapid entry into the brain from the c.s.f., which indicates that the interface between c.s.f. and brain is not impermeable to iodide, at least in one direction; it is not necessary for the validity of this theory that the membrane should be impermeable in the other direction. Thus, fluid relatively free of iodide flows into the brain from the

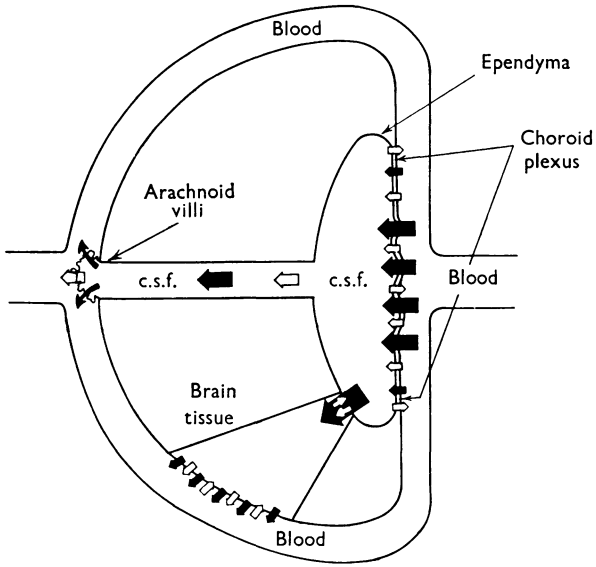

Fig. 16. A schematic representation of the central nervous system composed of blood, c.s.f. and brain tissue. The term 'blood' refers to the contents of the brain capillaries and venules and the term 'brain tissue' refers to the neural tissue interposed between the c.s.f. and the blood. Black arrows indicate bulk fluid flow from blood into c.s.f., from c.s.f. into brain tissue and also directly into the blood via the arachnoid villi, and from the brain tissue back into the blood. White arrows depict possible directions of solute movement. The small section of brain tissue, through which fluid flow and solute movement are depicted by the arrows, represents all brain tissue. The width of the black arrows indicates the relative rate of fluid flow from the c.s.f. into the brain and from the brain into the blood. This schematic presentation is not intended to show all fluid and solute exchange, but only the bulk flow of fluid and the movement of a solute which has a low c.s.f.: plasma ratio after I.V. injection. The direction and the magnitude of the solute movement are determined by the permeability characteristics of the membranes which must be crossed by the solute.

c.s.f., and iodide diffuses into the brain from the blood. It should be noted that the surface area of the brain capillaries is probably many times the surface area of c.s.f.-brain interface (Bakay, 1960; also see diagram, Fig. 16). Therefore the linear rate of flow of fluid into the brain from the c.s.f. must be many times that at which it leaves the brain to enter the blood, although the volume exchange would be the same. The diffusion of iodide

from blood to brain to c.s.f. would be opposed by an ever-increasing flow of fluid in the opposite direction as the iodide approached the c.s.f., and consequently the amount of iodide which actually enters the c.s.f. would be quite small. If the choroid membrane between c.s.f. and blood permitted a small amount of iodide to enter with the fluid being formed by secretion, then the c.s.f.:plasma ratio would increase. After I.C. injection iodide could enter the blood by flow through the arachnoid villi and by flow through the brain tissue; the bulk flow of fluid into the brain tissue would enhance the diffusion of iodide into the brain and then from brain into the blood. For substances such as iodide which are rapidly removed from the c.s.f. the major source of loss must be via the bulk flow of fluid through the brain tissue, while the important avenue of escape of substances which are slowly removed from the c.s.f. is via the arachnoid villi.

It can readily be seen that, given the proper combination of permeabilities of the membranes between blood and c.s.f., c.s.f. and brain, and brain and blood, this theoretical system could provide for the wide range of c.s.f.:blood and brain:blood ratios which have been reported in the literature, including those ratios which are only a small fraction of unity. The great complexity of the blood-brain-c.s.f. system, and consequently the large number of assumptions which would be necessary in constructing a mathematical model and the even greater difficulty of interpreting any results thus obtained, have hampered the development of such a model. For these reasons the volume of flow necessary for such a system to function is not known. However, the existence of this proposed mechanism is subject to experimental testing and such experiments are currently planned.

Each of the three tracers which entered the brain from the plasma had a different brain:plasma ratio or brain space. The brain iodide space was about 6% within minutes after injection and remained constant, inulin space was about 1·5–2% in 1–2 hr and also remained practically constant, and the sucrose space was 3–4% within 1 hr after which it increased steadily for the duration of the 43 hr experiment. After 43 hr the sucrose space was 19% and had not yet reached equilibrium. Are the tracer spaces related to specific physiological or anatomical spaces of the brain (extracellular, intracellular, glial, neuronal)? The fact that the iodide and inulin spaces remain constant at values which are only a small fraction of those one would expect if these substances distributed in total brain water is suggestive of an extracellular distribution. Similarly, the rapid initial rise in the sucrose space to about 4% followed by the sustained increase in space may reflect an initial distribution in the extracellular space associated with a slow entry into one or more brain cell types. The value of about 42% for the brain sucrose space after I.C. injection of the tracer,

when considered in conjunction with the constantly rising I.P. brain sucrose space, also suggests that sucrose is distributed intracellularly but probably not uniformly in all cells. If the I.C. brain sucrose space of 42 % included an extracellular space of 6 % (iodide space), the balance of 36 % appears to be of the proper order of magnitude for the neuronal or glial space. Experiments are currently in progress to attempt to establish whether the intracellular sucrose is associated primarily with glial cells, neurones, or both.

If the 2 % inulin space, 6 % iodide space, and the early 4 % sucrose space are reflexions of extracellular distribution of the tracers, the question arises as to why are the values not the same. In a dynamic system such as the C.N.S. the quantity of a tracer in the brain will be dependent upon the relative forces associated with entry and exit from the brain. For example, if bulk fluid flow through the brain tissue does occur, as the results reported here strongly suggest, this flow would oppose tracer diffusion into the brain from the plasma and would enhance movement from the c.s.f. into the brain. Owing to the much greater surface area of the capillaries compared to the area of the c.s.f.-brain interface (of the order of 10:1), the flow per unit area will be many times greater near the c.s.f. interface than near the capillaries, and the opposition to diffusion will also be much greater. This situation, in conjunction with variations in permeability between plasma and brain and also between c.s.f. and brain, could account for markedly different measures of the same extracellular space. Some of the factors that will affect the magnitude of the error in measuring the extracellular space with tracers in such a system are: (1) the extent to which the c.s.f.:plasma ratio varies from unity, since the fluid entering the brain from the c.s.f. will dilute the concentration of tracer in the E.C.F.; (2) the degree to which the tracer remains extracellular; (3) the permeability of the plasma-brain and c.s.f.-brain interfaces; and (4) the ability of the tracer to diffuse in the E.C.F. Thus, with a low c.s.f.:plasma ratio or a low c.s.f.-brain permeability, the fluid entering the brain from the c.s.f. would have a low tracer concentration and would tend to dilute the extracellular tracer. Similarly, if the permeability of the plasma-brain interface and/or the diffusibility of the tracer in the E.C.F. was low, the space as measured by the tracer would be much lower than the true extracellular space. An increase in the ratios, in the permeability, or in the diffusibility would reduce the difference between the tracer space and the true extracellular space.

Considering the many vicissitudes which make for difficulties in the measurement of extracellular space in brain, it is rather encouraging to find that the three values obtained were not more disparate. These results support the increasing body of evidence for the existence of a small but

real extracellular space in brain tissue. Although iodide has a low c.s.f.: plasma ratio, which would result in the iodide space yielding an underestimate of the extracellular space if bulk fluid flow exists, the rapidity of entry into and equilibration in the brain could compensate for some of this error.

SUMMARY

1. The nature of the blood-brain, blood-c.s.f., and c.s.f.-brain interfaces ('blood–brain barrier') was investigated by comparing the kinetics of movement and the tissue distribution of four isotopically labelled tracers in the rat. The tracer substances used, ^{131}I-iodide, ^{14}C-sucrose, ^{14}C-inulin, and radio-iodinated serum albumin (RISA), were selected primarily on the basis of their physical characteristics. The tracers were administered singly or in pairs either intracisternally (I.C.), intraperitoneally (I.P.), or intravenously (I.V.) and c.s.f., brain, muscle, and plasma samples were taken at various times from 2 min to 43 hr later.

2. Nephrectomized, 200–250 g, male Sprague-Dawley rats in groups of 8 or more were injected with 0·1 ml. of isotonic NaCl solution which contained 2–10 μc of the tracer substance. About half of each group was injected I.C. and the remainder I.P. or I.V. The radioactivity of each tissue was determined and the results were plotted as counts/min/g wet wt. of tissue, as a function of time after injection.

3. Iodide was found to distribute rapidly (about 5 min) in all tissues studied after either route of administration. After I.P. injection the tissue: plasma ratios were as follows: c.s.f., 0·03; brain, 0·06; muscle, 0·19. These ratios remained constant for the 43 hr duration of measurement. The movement of iodide between c.s.f. and plasma after administration by either route was extremely rapid compared to that of the other tracers studied.

4. The initial tissue:plasma ratios after I.P. administration of sucrose were as follows: brain and c.s.f., about 0·04; muscle, 0·13. In contrast to that of iodide, the brain:plasma ratio increased steadily during the 43 hr experiment, with a final ratio of about 0·19. The brain sucrose activity continued to increase even though the plasma sucrose level was slowly falling. The c.s.f.:plasma ratio reached a maximum of 0·10 at about 30 hr. The muscle:plasma ratio increased slightly during the experiment.

5. After I.V. injection of inulin the brain and c.s.f.:plasma ratios were 0·01–0·02, and increased only slightly during the 24 hr experiment. The muscle:plasma ratio was 0·12 and constant.

6. Following I.V. administration of RISA all the brain radioactivity could be accounted for by the residual blood and free iodide. The c.s.f.: plasma ratio was 0·01–0·015 with a maximum at about 8 hr. The initial

muscle:plasma ratio was about 0·02, and increased steadily during the 24 hr experiment to a value of about 0·04.

7. The results of the I.C. injections were as follows: (1) the time for the maximum brain radioactivity was less than 30 min for iodide, 2 hr for sucrose, 4 hr for inulin, and 6 hr for RISA; (2) the rate of decrease in radioactivity in the c.s.f. was RISA < inulin < sucrose < iodide; (3) although the early decrease in activity followed the above order there was a slower component to the c.s.f. falling-off curves which was common to RISA, inulin, and sucrose, and this may reflect loss through the arachnoid villi. Thus, the tracers other than RISA must be partly lost from the c.s.f. by routes other than via the arachnoid villi.

8. Some evidence has been presented to suggest that iodide is actively removed from the c.s.f. Possible mechanisms which might accomplish this have been discussed: these include a specific iodide pump and bulk flow of fluid from the blood into the c.s.f., from the c.s.f. into the brain tissue, and from the brain back into the blood.

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