Significance of Different Routes of Introduction on the Transport of Substances into the Brain[1] (35121)

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(Introduced by M. MAXWELL)
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When administering a substance that is not produced in the brain, its steady state concentration in the latter is a function of the rate of transport into and out of the central nervous system. Once passed the restrictions imposed by the blood–brain barrier, a substance may still be kept at a low concentration in its available space in the neural tissue by a continuous movement into the cerebrospinal fluid (CSF). Conversely, a substance introduced by way of the CSF may flow from brain to blood, thus again limiting its level in the tissue. This "brain drain" in either direction has been referred to as the sink action of the CSF and of the blood (1, 2). In order to estimate the true volume of distribution of a substance, defined as that theoretical space in which the measured solute is everywhere at the same concentration as in the perfusing media, it is necessary to block the sink actions described above, which can be done by simultaneously introducing the compound by blood and CSF.

These concepts were derived from studies with extracellular markers such as sucrose (3), inulin (4), sulfate (5), and iodide (6, 7), where diffusion is the main transport process involved in the movement of these substances across the fluid boundaries and in the depth of the tissue. In the case of iodide for which active transport processes were demonstrated across the choroid plexuses, the energy dependent component of the transport was blocked by using perchlorate as competitive inhibitor. In the present study we examine the influence of the postulated sink action on the behavior of two synthetic analogues, the amino acid 1-amino-cyclopentane-carboxylic acid (cycloleucine) and the carbohydrate 3-*O*-methyl-D-glucose that are known to be taken up but not metabolized by the brain cells.

Material and Methods. The experiments were performed in anesthetized New Zealand white rabbits of either sex, weighing between 2.5 and 3.5 kg. In addition to ^{14}C cycloleucine and ^{14}C methyl-glucose, ^{35}S sodium sulfate was used as an extracellular marker in order to calculate the intracellular concentration of the other two compounds. The specific activity of the ^{14}C isotopes was adjusted with cold carrier to 0.2 μCi/μmole and that of ^{35}S sulfate to 0.1 μCi/μmole and they were presented to the brain by either of the following ways: (a) brain cortical perfusion, (b) blood injection, or (c) the two above routes simultaneously. In order to prevent renal losses in procedures (b) and (c), kindneys were tied at the beginning of the experiments.

(*a*) *Brain cortical perfusion.* An artificial CSF reservoir was created by applying a plastic cylinder on the exposed surface of the brain, covering the two hemispheres. The leak-proof plastic rings was kept in place by an agar gel without exerting pressure on the pial surface (Fig. 1). Either a constant perfusion with a mechanical pump or periodic replacement of the solution kept constant the concentration of the materials (0.3 m*M* for the ^{14}C compounds and 0.5 m*M* for ^{35}S sulfate) throughout the 2 hr of exposure of the brain. Five percent CO_2 was bubbled in the

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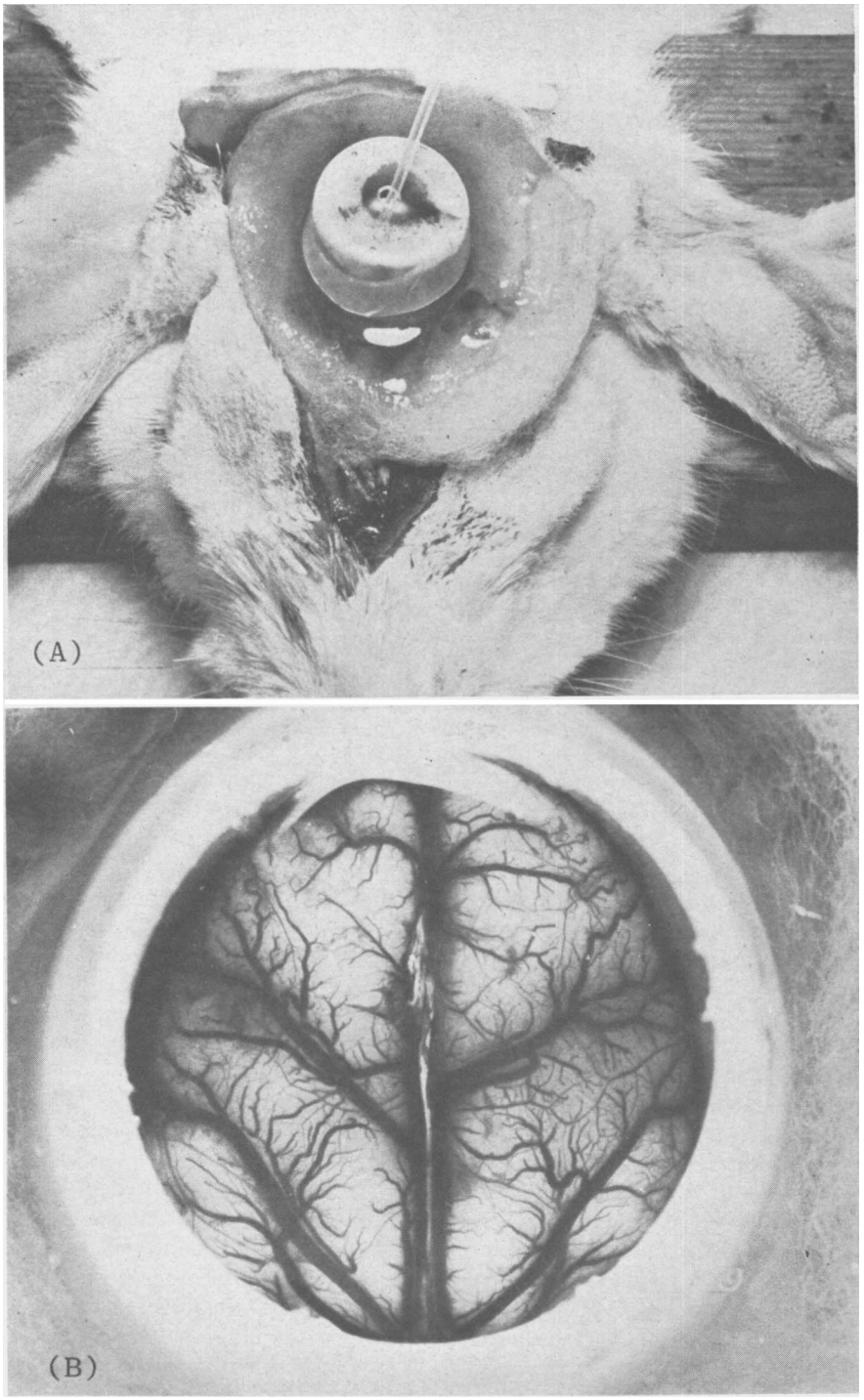

FIG. 1A. Brain perfusion using a plastic ring kept in position, leak-proof, by an agar gel. Five % CO_2–95% O_2 is bubbled through a polyethylene tubing piercing the cover of the ring. (B) Surface of both hemispheres inside the ring, bathed by artificial CSF.

reservoir to keept the pH of the perfusing medium in the range of 7.4–7.5 and to achieve a convenient mixing of the fluid.

(*b*) *Blood injection.* Approximately 5 μCi of one of the ^{14}C labeled compounds and of ^{35}S sulfate were administered together by a single intravenous injection. After the initial peak and rapid decay phase (30 min), blood concentrations decreased 10 to 20% throughout the next 2-hr period and the average of periodic blood samples was taken as the mean steady plasma value. This varied in different animals from 0.22 to 0.45 m*M* for the ^{14}C derivatives and from 0.20 to 0.61 m*M* for sulfate.

(*c*) *Combined cortical perfusion and blood injection.* After 30 min of an intravenous injection of approximately 5 μCi of the ^{14}C compound and 5 μCi of ^{35}S sulfate, a blood sample was withdrawn and counted. The concentration of the perfusion solution was adjusted to that of the blood values with a better than 15% accuracy. Brain was perfused for 2 hr.

At the end of the experiments the animals were decapitated, the whole brain was removed and placed on crushed ice in a humid chamber. The pia mater covering the upper cortical hemispheres was stripped off and the underlying surface was blotted with filter paper. A superficial slice, 400 μ thick from each hemisphere was cut with a calibrated slicer, weighed, and homogenized with 0.5 *M* perchloric acid. Aliquots of the supernatant were counted in a Nuclear Chicago liquid scintillation spectrometer in *p*-dioxane flour with an efficiency of 70%. The differential ^{35}C and ^{14}C counting were performed according to a previously described technique developed in the laboratory (8). The contribution of metabolites other than sulfate to the ^{35}S counts was eliminated by the use of perchoric acid as precipitant and by the use of barium chloride in the treatment of the supernatant for the differential counting. Samples of plasma and perfusion fluid were also processed accordingly. This resulted in the same quenching in all samples and no correction was necessary.

Paper chromatography in some of the brain extracts showed that the radioactivity was confined to the spot corresponding to the original compound.

To determine the contribution of residual blood to the measured content of labeled compounds in tissue, albumin-^{125}I was injected iv into 4 rabbits 10 min before sacrificing the animal. This contribution ranged between 0.7 to 1.2% and was subtracted from tissue values in procedures (b) and (c).

Results and Discussion. Our sulfate space values for each way of introduction are somewhat higher than those previously reported (5). Perfusion fluid in our experiments is in intimate contact with the external surface of the brain in contrast to artificial CSF in ventriculocisternal prefusion experiments. Factors such as dilution by newly formed CSF and tortuousity of the ventricular cavities are avoided with our technique. When the combined routes are used, sulfate space values (20.05%) closely approximate the addition of values obtained by each route separately (18.28%) (Table I). These results are consistent with the interpretation that a sink action is operative at both the CSF and the blood boundaries, decreasing the concentration in the tissue when the tag is given by either route alone. When the escape is prevented by the simultaneous administration by both routes, a more complete tissue "filling" occurs and a greater volume of distribution is obtained.

Tissue/medium ratio of cycloleucine is higher when given by blood than by subarachnoid perfusion ($p < 0.05$). Apparently there is no blood–brain barrier for cycloleucine as compared to the CSF side, although a time study would be necessary to establish if the velocity of penetration is the same by both routes. When cycloleucine is administered simultaneously by both blood and CSF, tissue/medium ratios are much higher than the sum of values obtained by each route separately. Active transport processes that were not apparent when cycloleucine was introduced by one route only, could become evident under the combined introduction and be responsible for the observed accumulation against a concentration gradient. Alternatively, a different intracellular compartment or a different cell population could be

TABLE I. Incorporation of Cycloleucine, 3-*O*-Methyl-glucose and Sulfate in Brain Tissue According to the Route of Administration.[a]

Compound	Blood injection		Brain perfusion		Blood injection and brain perfusion	
	cpm/ml of tissue H_2O / cpm/ml of plasma H_2O	intracel.[b] / plasma H_2O	cpm/ml of tissue H_2O / cpm/ml of medium	intracel.[b] / medium	cpm/ml of tissue H_2O / cpm/ml of medium	intracel.[b] / medium
Cycloleucine	1.20 ± 0.08 (12)	1.76	0.96 ± 0.04 (8)	1.33	3.22 ± 0.11 (12)	4.90
3-*O*-Methyl-D-glucose	0.54 ± 0.02 (16)	0.75	0.15 ± 0.01 (14)	—	0.65 ± 0.03	0.73
Sulfate[c] (%)	4.36 ± 0.43 (11)		13.92 ± 0.53 (13)		20.05 ± 0.77 (9)	

[a] Values are means ± SE. Number of determinations given in parentheses. Concentration of cycloleucine and of methyl-glucose in the medium, 0.3 m*M*.

[b] Intracel. = cpm per ml of intracellular H_2O, calculated from the equation: [(cpm tissue H_2O/cpm medium or plasma H_2O)—sulfate space]/(tissue H_2O—total sulfate space). Sulfate space in the numerator is the value of the respective column. Total sulfate space in the denominator is the value (20.0%) obtained by simultaneous blood and CSF administration. Tissue water is 81.6% of the tissue weight (10 determinations). Plasma water was taken as 94%.

[c] Sulfate values are expressed as space (ml per 100 g of wet tissue): (Tissue/plasma H_2O or medium) × 100 × 0.90. 0.90 is the correction factor to account for the Donnan distribution ratio between plasma and interstitial fluid (13).

more easily accessible when cycloleucine is introduced by the two combined sources. It has been suggested that two separate compartments could be reached by extracellular markers depending on the way of introduction (9). The maximal tissue/medium value of 2.63 for cycloleucine attained in our experiments, is much lower than that reported *in vitro* for slices of similar thickness: 13.0 (10). The damage introduced by slicing the tissue in the conventional *in vitro* procedure could be partly responsible for the high amino acid uptake obtained with brain slices (11).

Tissue/medium ratio of methyl-glucose when given by blood is higher than when introduced by CSF perfusion. Actually, the calculated space of distribution of methyl-glucose (0.15 × 100) obtained from CSF side is in the range of sulfate space by the same route (Table I). It is possible that the previously reported facilitated diffusion of methyl-glucose from blood to brain tissue (12) could also operate in the reverse direction. Therefore, methyl-glucose coming from the perfusion fluid would be drained to blood at a faster rate than the rate of entry. This would prevent filling up of total extracellular space (20.0%). When the two routes are used simultaneously, contribution of the blood source predominates and the tissue uptake is of the same magnitude as when this latter route is used alone. Thus, the CSF contribution for this compound is relatively unimportant when compared to penetration from blood.

In conclusion, the results presented here are compatible with the following interpretations: (i) The rate of entry of a given substance into the brain can vary widely according to the route of introduction. The use of the three described procedures can disclose the relative importance of the different routes; (ii) For certain compounds, like cycloleucine, transport processes otherwise not apparent could become active when escape from the brain tissue is prevented by simultaneous administration by blood and CSF; and (iii) If more than one compartment in the brain is accessible to a substance, different routes of introduction may provide preferential access to each one of them.

Summary. Sulfate, cycloleucine, and 3-*O*-methyl-glucose were introduced into the brain by three different routes: (a) brain perfusion, (b) blood injection, and (c) blood injection and blood perfusion simultaneously.

The value attained in the nervous tissue with sulfate, which is considered an extracellular marker, when administered by the two combined routes, was equal to the addition of data obtained by each route separately. Using the combined procedure (c) for cycloleucine, a higher value than that calculated from the sum of procedures (a) plus (b) was reached. The data of the combined introduction for methyl-glucose were in the range of those obtained by blood injection alone.

The significance of different routes of administration on the uptake of substances into the brain is discussed.

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