## Disappearance of Different Substances in Contact with the External Surface of the Brain

*Introduction.* The behaviour of substances introduced into the cerebrospinal fluid (CSF) in relation to their absorption by the nervous parenchyma is a matter of controversy.

The penetration of substances such as sodium, urea, $^{131}$I-albumin, phosphate and others into the nervous tissue is considerably faster from the CSF than from the blood[1–4]. Dealing with amino acids, an ample distribution and increase above control levels was shown for leucine and lysine when given by subarachnoid route[5]. The brain incorporates more radioactivity when $C^{14}$ proline and methionine are injected intracisternally than when they are intravenously administered[6–7].

On the other hand, there are several references opposed to the concept of free passage of substances from the CSF to the brain tissue. Intracisternally-administered glucose is unable to correct experimental insulinic hypoglycaemia[8]. ERNSTER and HERLIN[9] postulate an active incorporation of labelled phosphate injected intracisternally into the ependymal and leptomeningeal layers with a restriction of the penetration into deeper areas. BAKAY[10] has proved that when $^{32}$P phosphate was kept 55 min in contact with the external surface of the brain only 30% was found in the tissue. KLATZO et al.[11], with fluorescent protein by intraventricular injection, obtain very little passage into the adjacent layers when the tissue is not damaged by the experimental procedure. When injected intravenously, triiodothyronine is distributed in the brain, but by cisternal administration retention by the pia presumably occurs[12]. In our laboratory we have shown in cats, a restriction of the passage of amino acids from a ventricular perfusate into the nervous parenchyma[13].

Because of the anatomical differences between the ependymal and pial linings, we decided to study separately the ventricular and subarachnoid compartments, instead of using single injections for the whole cerebrospinal fluid space. In this paper we are dealing with the external subarachnoid lining.

*Material and methods.* A technique based on the implantation of small plastic cylinders (8 mm diameter) on the pial surface was used[14]. In this way a cup is formed into which different substances dissolved in artificial CSF[15] can be introduced. The solution is in contact only with the brain surface underlying the cup without interference from other variables (flow of CSF, varying space between meninges, uneven distribution of the solution etc.). Adult cats of either sex under nembutal anaesthesia were used throughout. The cups with the substances under study were left in contact with the pial surface for times ranging from 2 to 5 h according to the substance. In all cases time was constant for each substance. Samples were


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Table I. Percentage of recovery and T $^1/_2$ of disappearance of different substances in contact with the brain surface[a]

| | | Time in min 10 | 20 | 30 | 60 | 120 | 180 | 240 | 300 | T $^1/$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Ethyl alcohol | | | | 60.6 ± 5.2 | 45.0 ± 6.3 | 24.0 ± 4.2 | | | | 50 |
| Ethylthiourea[b] | | | | 84.3 | 78.1 | 51.0 | 41.7 | | | 130 |
| Urea | $C^{14}$ | 99.5 ± 7.9 | 97.7 ± 8.9 | | 68.5 ± 6.4 | 53.9 ± 3.3 | 45.8 ± 8.1 | 34.1 ± 3.5 | 30.0 ± 3.8 | 140 |
| Glucose | $C^{14}$ | | | | 94.5 ± 3.9 | 83.2 ± 1.3 | 76.7 ± 4.1 | 57.3 ± 3.3 | 55.8 ± 3.3 | 378 |
| Leucine | $C^{12}$ | | | | 93.7 ± 9.6 | 78.3 ± 6.9 | 68.0 ± 9.2 | 61.6 ± 6.9 | 55.0 ± 9.8 | 320 |
| | $C^{14}$ | | | | 86.5 ± 8.6 | 74.8 ± 6.5 | 70.4 ± 6.0 | 61.9 ± 3.3 | 49.1 ± 5.1 | 300 |
| GABA | $C^{12}$ | | | | 91.5 ± 6.8 | 81.5 ± 5.3 | 77.2 ± 4.1 | 62.8 ± 4.6 | 60.6 ± 6.7 | 405 |
| | $C^{14}$ | | | | 96.9 ± 6.8 | 78.5 ± 6.0 | 74.9 ± 6.9 | 63.9 ± 6.4 | 57.9 ± 6.5 | 387 |
| Glutamic acid | $C^{12}$ | | | | 91.6 ± 6.2 | 79.6 ± 4.3 | 71.4 ± 4.1 | 61.8 ± 2.3 | 57.5 ± 7.8 | 366 |
| | $C^{14}$ | | | | 91.8 ± 3.5 | 87.0 ± 6.6 | 69.5 ± 8.1 | 52.4 ± 1.9 | 50.5 ± 5.6 | 309 |
| Lysine | $C^{12}$ | | | | 93.3 ± 5.1 | 85.3 ± 6.1 | 81.6 ± 6.1 | 72.8 ± 4.8 | 67.5 ± 6.9 | 548 |
| | $C^{14}$ | | | | 97.0 ± 3.5 | 94.1 ± 4.1 | 80.5 ± 4.1 | 77.5 ± 6.7 | 70.0 ± 4.5 | 631 |
| Phenylalanine | $C^{12}$ | | | | 89.7 ± 4.6 | 86.1 ± 4.7 | 82.0 ± 6.2 | 77.6 ± 7.6 | 67.5 ± 8.3 | 498 |
| | $C^{14}$ | | | | 102.6 ± 4.0 | 86.5 ± 3.9 | 80.1 ± 6.5 | 78.8 ± 11.2 | 70.3 ± 6.3 | 570 |

[a] Samples taken from the container on the pia. Initial concentration: 100%. Results ± standard deviation of 3 to 12 determinations. [b] Mean of 2 determinations for each point.

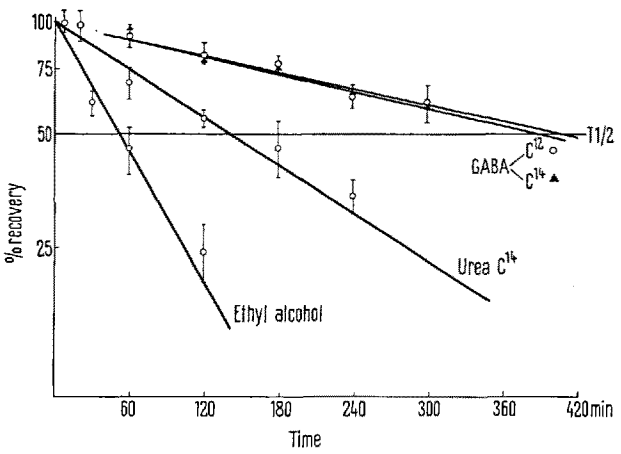
taken at 60 min intervals, except for the first hour when 10–20 min samples were collected for urea and 30 min samples for ethanol and ethyl thiourea. The decay in concentration for each substance and for each cup was followed and results are expressed as % of recovery in the cup or in terms of half-life ($T^1/_2$).

The initial concentration of amino acids in the cup was 10 m*M* and they were used with their corresponding $C^{14}$ isotopes. The $C^{12}$ amino acids were quantitatively estimated by paper chromatography according to Cook and Luscombe[16]. Ethyl alcohol (initial concentration in the cup: 10 m*M*) was determined by the microdiffusion technique of Conway and Nolan[17], and ethyl thiourea by the method of Grote[18]. $C^{14}$ labelled urea and glucose were used, the carrier concentration being 2.2 m*M* for urea and 3.0 m*M* for glucose. The $C^{14}$ counting was performed in planchettes in an automatic gas flow counter (N. Chicago).

In some cases, $C^{14}$ determinations of amino acids were performed in the nervous parenchyma underlying the cup. The tissue was excised immediately after taking the last sample from the cup (5 h of contact), rinsed with cold saline, blotted on filter paper and frozen. Then it was sectioned in 3 portions 3 mm thick from the pial surface inwards. After homogenization with cold ethyl alcohol 80%, aliquots of the supernatant were taken for $C^{14}$ counts.

*Results and comment.* From results presented in Table I it can be seen that there are different times of disappearance for the assayed substances. Amino acids show a comparatively slow rate of disappearance and since the half-life of their $C^{12}$ and $C^{14}$ isotopes is not significantly different, an exchange diffusion mechanism could be excluded.

The Figure shows the curves of disappearance for three types of substances: ethyl alcohol as a lipid soluble material; urea as a highly diffusible water soluble compound; and γ-aminobutyric acid (GABA) as a compound which is metabolized by the brain and actively accumulated against concentration gradients by brain tissue slices. From the curves it can be deduced that the disappearance of these substances follows a first order kinetic. The rest of the assayed substances also gave curves of a similar type with different slopes according to the compound. The lipid solubility appears as a factor favouring the passage of substances, which is in agreement with concepts of transport across membranes[19], although this point of view is not totally accepted by others in the case of CSF-brain interrelationships[20].




Curves of disappearance of some of the substances in contact with the brain surface. Values taken from Table I. for $C^{14}$ GABA (▲) standard deviations are not presented for the purpose of clarity.

According to the high uptake of amino acids by the nervous tissue as shown by in vitro studies[21], and also the ability of this tissue to metabolize different amino acids in vitro and in vivo[22], one would expect a high rate of disappearance of these compounds from the cup into the nervous parenchyma. However, our results suggest that in the external surface of the brain there exists a barrier or restriction of the penetration of amino acids into the nervous parenchyma.

In Table II, results from determinations in the tissue are presented. It can be seen that the distribution is not homogeneous, and that in the more superficial section the concentration ranges between 8.5 and 27.4% of the initial concentration in the cup. Of the total $C^{14}$ counts found in the tissue, 87 to 95% of the counts were in the more external 3 mm layer. This distribution is of the type mentioned by Ford[12] for triiodothyronine which is retained in the pia. It is a further indication of the restriction of the passage of substances into more internal layers of the brain tissue[23].

Table II. Passage of $C^{14}$ amino acid from the external surface to the brain parenchyma. % $C^{14}$ recovery in the tissue[a]

| Amino acid | 0–3 mm section (pia-cortex) | 3–6 mm section | 6–9 mm section |
|---|---|---|---|
| GABA | 27.4 | 1.3 | 0.5 |
| Glutamic acid | 8.5 | 0.5 | 0 |
| Lysine | 14.0 | 1.2 | 0.7 |
| Leucine | 16.0 | 0.5 | 0.4 |

[a] Initial concentration in the cup: 100%. Each value is the mean of three determinations.

*Résumé.* La disparition de différentes substances en contact avec la surface externe du cerveau est étudiée. Les temps moyens de disparition de celles-ci suggèrent l'existence d'une barrière s'opposant au passage de ces substances du compartiment subarachnoïde crânien au tissu nerveux.

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