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BRAIN PROTEIN SYNTHESIS IN NORMAL AND DEMENTED PATIENTS

- A study by P.E.T. with ^{11}C-L Methionine -

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Brain proteins, dementia, T.E.P., ^{11}C Methionine

Introduction

Positron Emission Tomography (P.E.T.) provides us the original opportunity for in vivo study of brain protein synthesis in man. This particular function seems to have an unique behavior in nervous tissue through some diseases and a variation in one of its physiological parameters is not always refound with other metabolic studies with FDG or $^{15}O_2$.

So we have developped a dynamic three-compartment model of protein synthesis in brain (Fig. 1)(1). Using collection of cerebral activity data by P.E.T. after a bolus injection of ^{11}C-L-MET and factorial analysis, we found that our results were explicited by such a three compartment pathway. This model was also carefully checked by varied biochemical and physiological procedures in rats and baboons. It was finally validated for animal quantitative autoradiographic studies of protein synthesis in brain.

Briefly, the biochemical basis for choice of Methionine are as follow :

- Being an indispensable amino acid, it penetrates readily through the blood brain barrier. During the course of the kinetic study, which is limited to 45 minutes due to the short half-life of ^{11}C (T = 20.4 min), only a negligible amount of methionine follows the transmethylation pathway (7, 8) : less than 2 % during one hour in baboons.
- The labeled amino acid traces the total incorporated methionine since no de novo synthesis and very little recycling takes place (9).
- Methionine may be easily labeled at high specific activity (600 Ci/mM) on the methyl group in the natural L form (2) which is the one incorporated into proteins (5).

Direct final checking of our method was realised on 4 baboons Papio papio (6 to 8 kg weight) anesthetized by ketamine 2 mg/kg. Scheduled injections of ^{3}H, ^{14}C or ^{11}C Methyl labeled L-Methionine were done during P.E.T. recording

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of C^{11} brain radioactivity. At given times up to 45 minutes after first injection the baboons were sacrified and samples of brain homogenates, trichloracetic acid and organic solvants precipitates (protein fraction) and collected supernatants (free amino acid or metabolic derivatives fraction) were measured for ^{11}C, ^{3}H and ^{14}C radioactivities.

In vivo brain radioactivity was corrected for blood activity contribution after measuring the day before cerebral blood volume using $^{68}GaCl_3$ as a blood protein marker (6). Arterial blood samples were withdrawn at given times for radioactivities and cold methionine measurements. Fig. 2 shows the good agreement between the curves obtained for each fraction applying the model to the in vivo experimental data in the baboon and the in vitro measurements of radioactivity in these fraction (TCA-solvants precipitate, supernatant and total homogenate). This confirms our biochemical and computationnal experiments and validates the three compartment model with L-Methionine as an approach for measuring amino acid brain incorporation. It is shown that, 45 minutes after injection, 50 % of the methionine tracer dose present in the brain is incorporated into the protein pool.

Direct chronical samplings from ventricules or cisterna magna of 10 baboons, even 1 hour after ^{11}C-Met injection never showed any cerebro-spinal radioactivity superior to 1/100 of blood activity and so,much less than brain tissue activity, confirming exclusion of brain cavities from our compartmental model (see also human brain images).

We report here its application by P.E.T. to 48 patients suffering or not Alzheimer's type dementia.

Principle and Methods

Briefly, the patient's head is positionned under an ECAT II Ortec camera

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at orbito-meatal + 5 cm level. Spatial resolution is 19 mm axialy and 16 mm transversaly. Special care was given to quantification through daily activity standardisation of the camera, transmission data for picture reconstruction, hardware correction of random coïncidences, ...

Pure C^{11}-L Methionine (600 Ci/mM) was injected rapidly intravenously in the patient's arm (20 mCi within 2-3 ml) and blood activity during scanning was sampled in arteriolized venous blood. Corrections were introduced for the difference between cerebral and peripherial hematocrit (0,85) as for red cell versus plasma activity (0,66 in volume).

Twelve scans are recorded : 6 x 1', 4 x 5', 2 x 10' with at least 800,000 events per image. Overall duration for metabolic examination is 47 minutes during which the patient is rested in warm and quiet conditions.

For a precise calculation of the most important parameters of protein metabolism in small regions of brain, a correction for residual blood activity in the slice is needed. This is done the day after by a 10 minute image of the same slice level using $^{68}Ga\ Cl_3$, transferrin label, as a plasmatic marker (Fig. 3). Blood sampling allows direct calibration of the picture in ml blood/pixel or ml brain. An iterative program of modelisation using the cerebral blood activity curve and corrected brain activity curve yields the 3 transport constants of the model and so calculates activity in the two brain compartments : free methionine in extra-cellular space and incorporated into proteins (Fig. 4).

The final images for computer calculation were a serie of 12 scans corrected for cerebral blood activity, which was high in early images : still 15 % for example 5 minutes after intravenous injection of ^{11}C-L Methionine. For each pixel of a transformed 50 x 50 image, a 12 points curve is extracted and fitted. Local parameters are given numerically and as real metabolic maps of the slice : methionine input and partition coefficient at the blood brain

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barrier level, half-life of free methionine and protein incorporation rate are the main clinically demonstrative parameters (see figure 5, 6).

The mean age of our demented subjects (N = 28) was 86.3 ± 6.1 years, 80.2 ± 9.4 years for the normal group (N = 20). All were right handed.

Clinical, biochemical and psychometric tests (W.A.I.S. and Rey's test) were done on each patient. Demented patients had not been treated for three weeks. A C.T. scan completed the examination set and gave valuable data about the local anatomy of the studied slice. Particular efforts were made to avoid any form of vascular dementia (clinical history, Hachinski's test, C.T. scan, ...). Lacking some results at the present time, we have classified demented subjects roughly in two groups according to clinical and psychometric tests results. Three of our very demented patients were discarded because they showed brain abnormalities in their C.T. scans compared to the cross-matched normal reference group.

Results and Comments

Figure 4 shows a ^{68}Ga image of cerebral blood volume in dementia. As others (4), we have not found any local or global modification of this volume in demented patients even in a more altered state : Mean CBV : 5,4 ± 0,5 ml blood/100 g grey matter and 3,0 ± 0,3 ml blood/100 g white matter. On the other hand, important modifications of local protein synthesis were found in these cases.

Besides the absolute values of brain physiological parameters, we calculated an occipital to frontal ratio using large bilateral symetric zones on metabolic maps according to the anatomy of the brain slice studied.

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Alzheimer's type dementia

Metabolic maps given in figure 6 are compared to normal values in figure 5. The results are summerized in tables 1 and 2.

Regarding the clinical status, a net diminution in frontal metabolism can be seen in Alzheimer's type dementia. This is particularly emphasized by calculating the fronto-occipital ratio in the studied brain slice. Parietal cortex is also often altered and the reason for a relative apparent stability of occipital tissus in the disease is not known but was also observed with FDG studies (3). A decrease of more than 65 % in the protein synthesis rate has been measured in the most severe Alzheimer's type demented patients.

Preliminary and partial results about psychometric tests, which are still on the way, correlate very well with this over-cited measurements of protein synthesis function in brain.

Conclusion

Protein metabolism is very stable in the brain and not correlated with any acute stimulation of neurons. Comparison with C.T. scans shows that a metabolic impairment precedes by some time all signs of death and disparition of cerebral tissue. Dramatic variations can be found in protein metabolism even in light dementia and it is likely that, as reported for other kinds of cells, a decrease in protein metabolism is a normal reaction of the nervous cells to biochemical injury. Discordance between glucose and amino-acid metabolism in focal lesions such as epileptic foci or cortical injuries in animals (10, 11) confirm the interest and the originality of protein synthesis for itself in the brain.

Much remains to be learned about protein synthesis in the brain and C^{11}-L-Methionine examination of human brain with P.E.T. can improve our understanding of mental illness.

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Table 1 : Whole slice MET metabolism in dementia

	Normal	Dementia
Input	0.46 ± 0.16	0.31 ± 0.12
Half-life	8.1 ± 1.9	9.0 ± 2.7
Incorporation	0.15 ± 0.05	0.09 ± 0.04
Extraction	24.7 ± 9.4	14.9 ± 5.7
	N = 20	N = 25

Input & Incorporation : nM/min/gr brain

Half-life of free Methionine : minutes

Extraction : %

Normal : 80.2 ± 9.4 y , Dementia : 86.3 ± 6.1 y

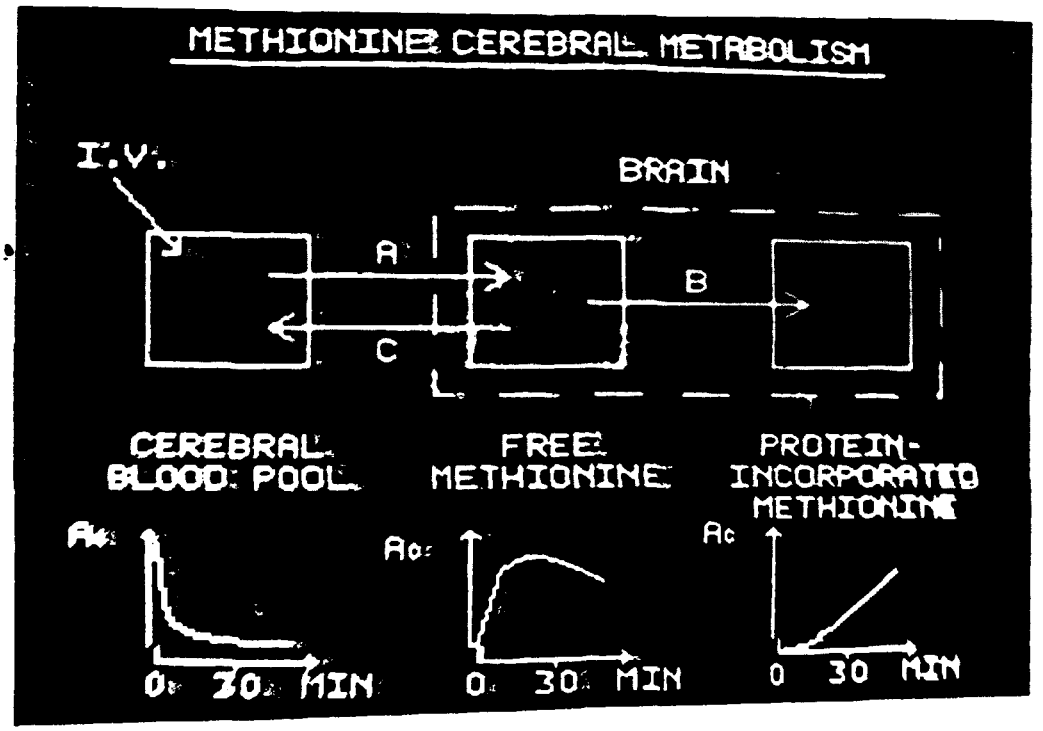
Table 2 : Local protein synthesis, fronto-occipital ratio

R.O.I.	Normal [a]	Dementia [a]
Frontal	0.15 ± 0.06	0.09 ± 0.03
Occipital	0.16 ± 0.06	0.15 ± 0.07
F/O ratio	0.96 ± 0.11	0.64 ± 0.21
	N = 20	N = 25

R.O.I.	Light dem. [a]	Severe dem. [a]
Frontal	0.13 ± 0.03	0.05 ± 0.02
Occipital	0.15 ± 0.05	0.10 ± 0.04
F/O ratio	0.99 ± 0.18	0.52 ± 0.13
	N = 8	n = 17

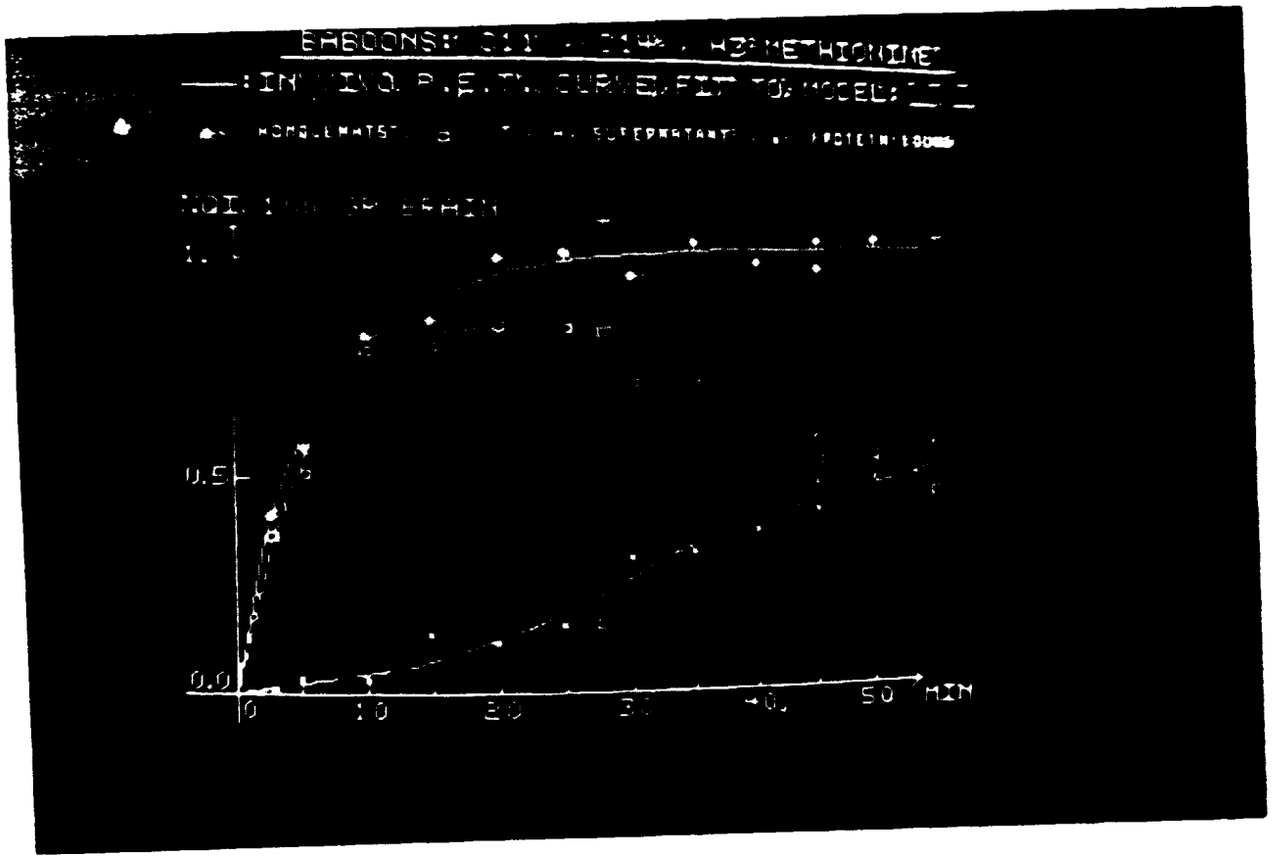
[a] lCMR methionine : nM/min/gr brain. OM + 5 cm level.

Fig. 1 : Three-compartment model of Methionine metabolism in brain following intravenous bolus injection



Assuming a mean half-life for cerebral proteins of around 14 days, no significant ^{11}C activity can be refound in degradation products of newly synthetized proteins during the 45 minutes of examination. So B constant alone represents protein incorporation of ^{11}C Met in brain.

Fig. 2 : In vivo and in vitro measurements on baboons



T : total brain activity
F : free ^{11}C methionine in brain
P : Protein incorporated C^{11}-methionine

Fig. 3 : Cerebral blood volume map with $^{68}GaCl_3$

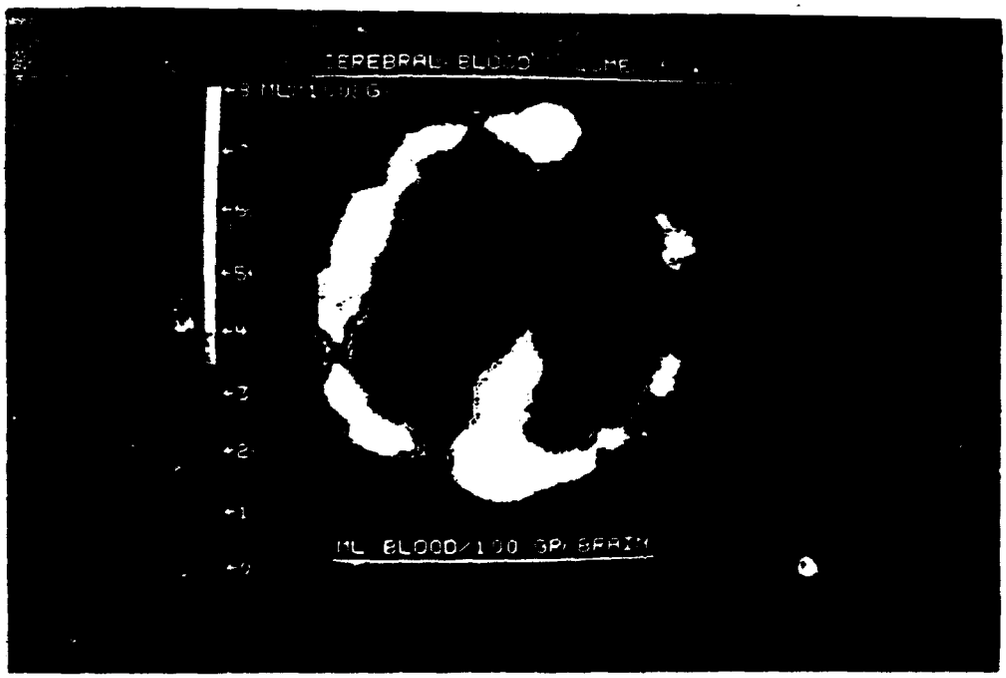


Fig. 4 : Computer fitting of experimental data

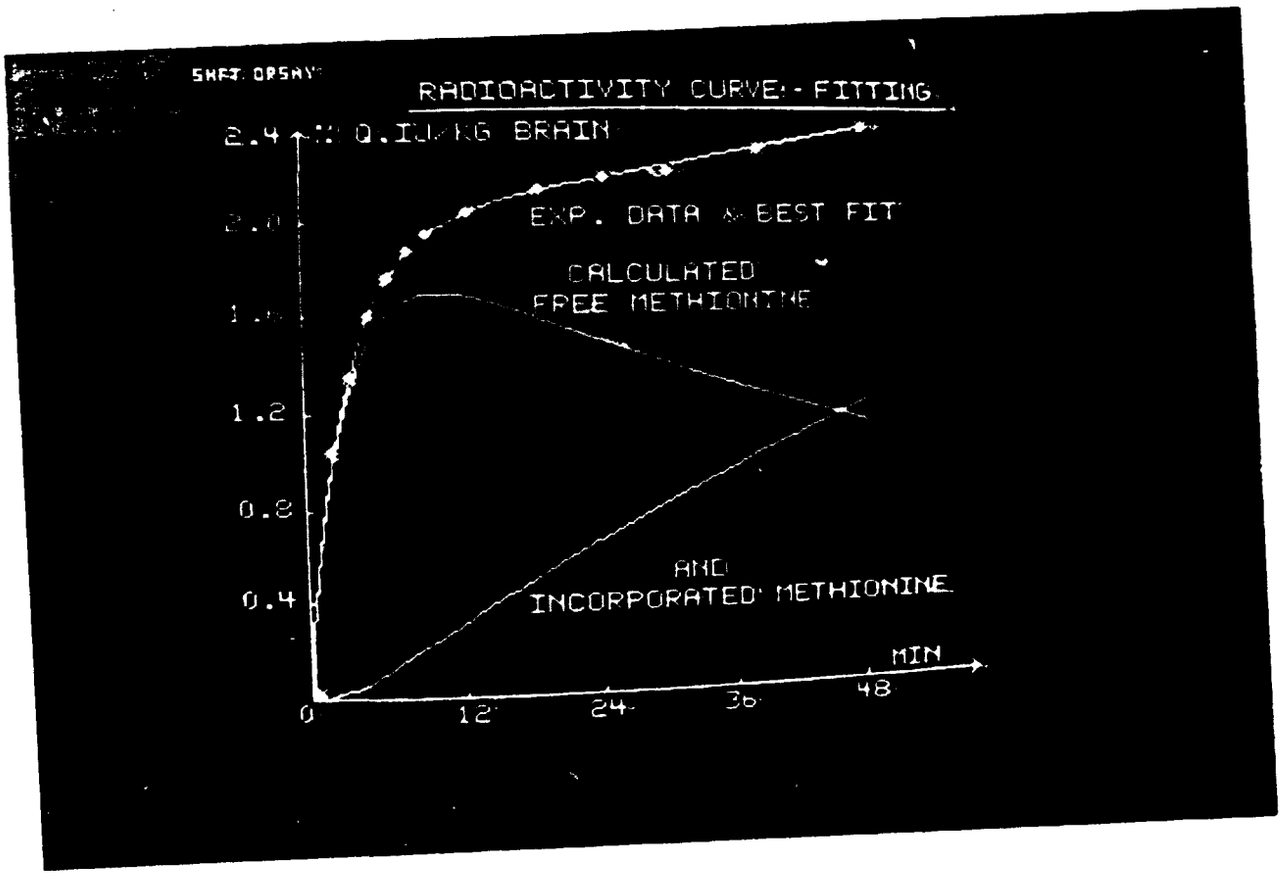


Fig. 5 : Metabolic maps at OM + 5 cm level in normal patient

Input and Incorporation in nM/min/gr brain (see tables 1, 2). Half-life in minutes. Partition coefficient of methionine between brain spaces and blood remains very constant in each case reflecting well statement of blood-brain-barrier. (All the color scale is used for each image and is not related to compared absolute value in the two cases. See figure 6).

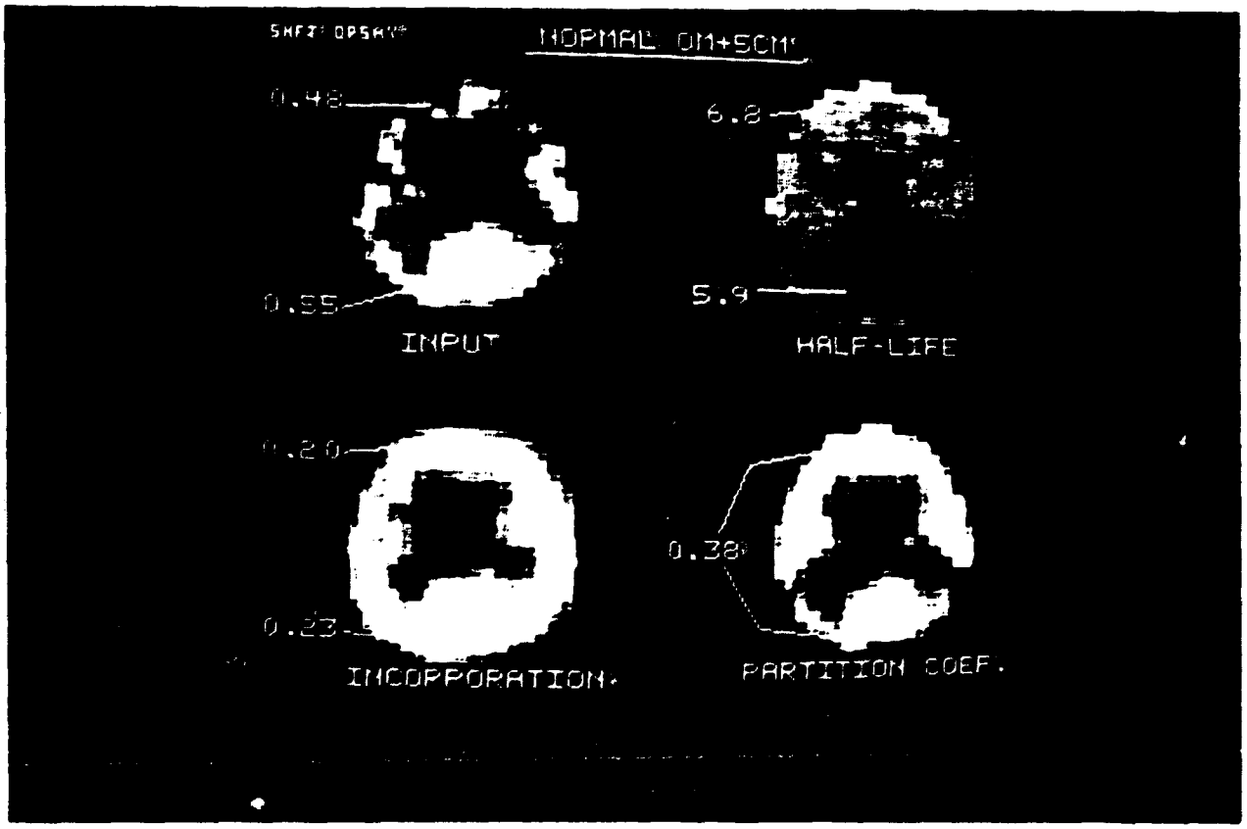
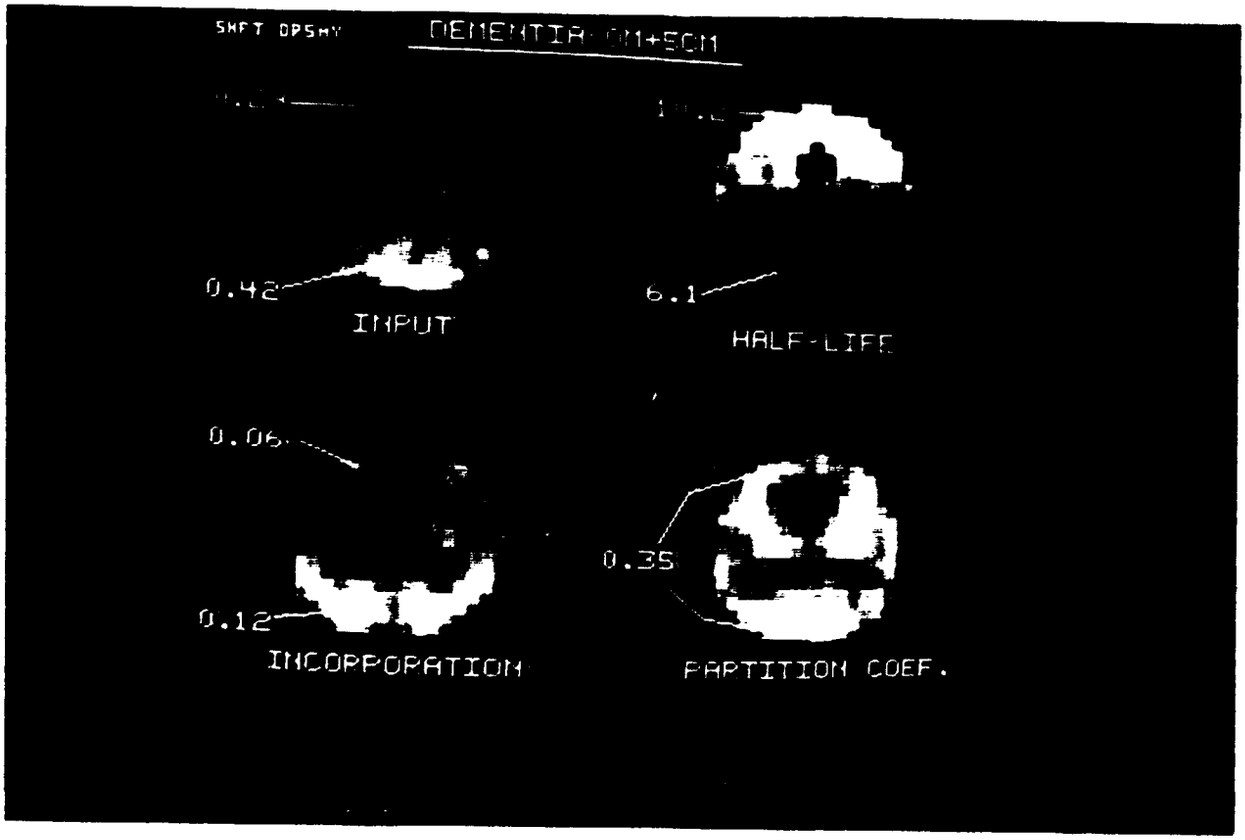


Fig. 6 : Metabolic maps at OM + 5 cm level in an Alzheimer's type demented patient (units see Figure 5).



R E S U M E

P. BUSTANY, J.F. HENRY, F. SOUSSALINE, D. COMAR.

"Brain protein synthesis in normal and demented patients : A study by P.E.T. with ^{11}C-L-Methionine".

La validation pratique d'un modèle compartimental représentatif de la synthèse protéique dans le cerveau a été effectuée chez 9 babouins. Des injections séquentielles sur le même animal de méthionines C^{11}, ^{3}H, ^{14}C, suivie de l'enregistrement par T.E.P. de l'activité γ dans une coupe cérébrale choisie au cours du temps ont permis de mesurer par broyage et précipitation au T.C.A. la répartition de la méthionine injectée dans les différents compartiments du modèle après un bolus. La superposition des résultats directs in vitro à ceux calculés par ordinateur est excellente.
Cette technique d'étude de la synthèse protéique cérébrale in vivo a été appliquée chez 28 déments de type Alzheimer et 20 normaux de même âge. La corrélation entre les résultats des tests cliniques et psychométriques et l'activité de synthèse protéique cérébrale confirme une anomalie de cette voie biochimique de synthèse au cours de la maladie. Une baisse d'activité de 65 % peut se rencontrer dans les lobes frontaux de certains malades !

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