ENZYMATIC SYNTHESIS OF ^{11}C-LABELLED S-ADENOSYLMETHIONINE

Paul GUEGUEN, Jean-Louis MORGAT

Service de Biochimie
Département de Biologie
Centre d'Etudes Nucléaires de Saclay
B.P. 2, 91190, Gif-sur-Yvette - France

Mariannick MAZIERE, Gérard BERGER and Dominique COMAR

Service Hospitalier Frédéric Joliot
Département de Biologie
Centre d'Etudes Nucléaires de Saclay, Orsay - France

Michel MAMAN
Institut Choay
10, rue Morel - 92220, Montrouge - France

SUMMARY

This article describes the radiosynthesis of ^{11}C-labelled S-adenosylmethionine by an enzymatic process. This methyl donor was prepared by condensation of L-(methyl-^{11}C) methionine with ATP. The synthesis and purification could be carried out in 20 minutes with a final yield of 80 %. The specific radioactivity obtained was close to 200 Ci/mmole. 6 mCi of ^{11}C-labelled S-adenosylmethionine was injected in a rabbit, and its repartition followed by an opticamera.

Key words : SAMe, ^{11}C-labelling, scintigraphy

0362-4803/82/020157-14$01.40

Received February 11, 1981

INTRODUCTION

The short lived radioisotope ^{11}C ($T_{1/2}$ = 20.4 min) is a positron emittor / 1 / of considerable potential interest for physiological and pharmacological studies on whole organisms, since it can be detected by external devices / 2, 3, 4, 5, 6 /.

These are two difficulties associated with ^{11}C chemistry. One is the requirement of rapid conversion steps between the ^{11}C labelled starting material and the final expected compound, the second difficulty concerns the handling of very minute amounts of ^{11}C labelled compound of the order of 0.01 to 0.2 µmoles, since their specific radioactivities are in the range of hundred of Curies per mmole.

Therefore classical synthetic procedures or purification schemes have to be adapted to overcome the specific difficulties raised by ^{11}C handling.

In our present field of interest we wished to label with ^{11}C catecholamines and neuro-modulators and were led to consider labelling Nor-derivatives by enzymatic methylation. The first step to reach was therefore the capacity to prepare [^{11}C-methyl] S-adenosyl-methionine.

The easiest way to obtain S-adenosylmethionine is to condense enzymatically L-méthionine and ATP. CANTONI et al. / 7-8 / gave a thoroughly detailed description of S-adenosyl-methionine preparation. Further studies of this topic were carried out also by CAILLARD / 9 /, MATTHYSSE et al. / 10 /,

SCHLENK et al. / 11 /. Whereas the yields reached by these procedures were satisfactory, the time-span required were incompatible with the short half-life of ^{11}C. We were therefore led to re-investigate the published procedures under the headings of time economy. The results, a complete preparation in 20 minutes, starting from inorganic ^{11}C, are described in the present paper.

1 - MATERIALS

ATP was purchased from Boehringer Manheim (GFR). Non-labelled methionine was from Merck (GFR) and L-[methyl-^{14}C] methionine was prepared by Commissariat à l'Energie Atomique (France) with a specific radioactivity close to 55 mCi/mmole.

S-adenosyl-methionine chloride was obtained from I.C.N. (USA) and dithiotreitol from calbiochem (USA).

Ion exchange resins AG 50 WX_4 (NH_4^+ form), $AG1X_8$ (carbonate form) and gel permeation Bio-Gel P-10 were provided by Biorad Laboratories (USA).

All other chemicals and solvents were of analytical grade from Prolabo or Merck.

The L-methionine-S-adenosine transferase (EC2-4-2-13) extracted from rat liver by a method adapted from LOMBARDINI et al. / 12 /, was kindly supplied by Choay Institute (Paris).

S-adenosyl-methionine biosynthesis and purification was quantified by use of the ^{14}C labelled derivative, associated with high performance N-layer chromatography (HPTLC) plates (Silicagel 60 F.254 Merck), and HPLC (Waters : pump 6000, injector U 6K and UV detector M 440) on partisil SCX columns (Whatman). Radioactivity was detected with an ionization chamber. Absorption spectra were carried out on Beckman model DK 2 A.

Radio-scans of thin-layer chromatographic plates were performed with a Berthold scanner II. Autoradiography was achieved on RPX-O mat medical X-ray film (Kodak).

2 - METHODS

2.1 - Enzymatic purification of methionine-adenosyl-transferase

The preparation of the méthionine-adenosyl-transferase (MAT) followed the procedures described by CANTONI and DURELL / 8 /, LOMBARDINI / 12 / and PAN en TARVER / 13 /.

From male Wistar rats, weighing approximately 200-250 g, 20 g of liver were obtained and homogenized in 5 volumes of phosphate buffer (0.03 M ; pH : 6.9), stirred during 15 minutes and then centrifuge at 18,000 X g for 30 minutes.

The supernatant was fractionated with saturated NH_4 sulfate (pH : 7) the fraction precipitating between 33 and 50 % saturation was then dissolved in 20 ml Tris buffer (pH 7.8) 50 mM, KCl 50 mM, $MgCl_2$ 5 mM, 2-mercapto-ethanol 5 mM and applied for desalting on a Sephadex G 25 column (3 x 20 cm) previously equilibrated with the same buffer. The eluted proteins (30 ml) were then applied on a DEAE-cellulose column (3.5 x 25 cm) equilibrated in the same buffer containing 20 % of glycerol.

Elution pattern was obtained with a linear gradient of KCl 50 mM to 500 mM in the same buffer described above. The eluted fractions containing significant quantities of enzyme activity was eluted between 0.20 to 0.30 M KCl (figure 1). The specific activity of enzyme is approximately 2 units/mg of protein and represents a 33 fold purification with a recovery of 40 % of the initial enzyme activity.

A unit corresponds to the transformation of 1 nmole of substrate per minute per mg protein under specified conditions / 9 /. The enzymatic preparation was stored in : 50 mM Tris pH 7.5 ; 5 mM $MgCl_2$; 5 mM β-mercaptoethanol ; 200 mM KCl and 20 % v/v glycerol, and frozen in liquid nitrogen.

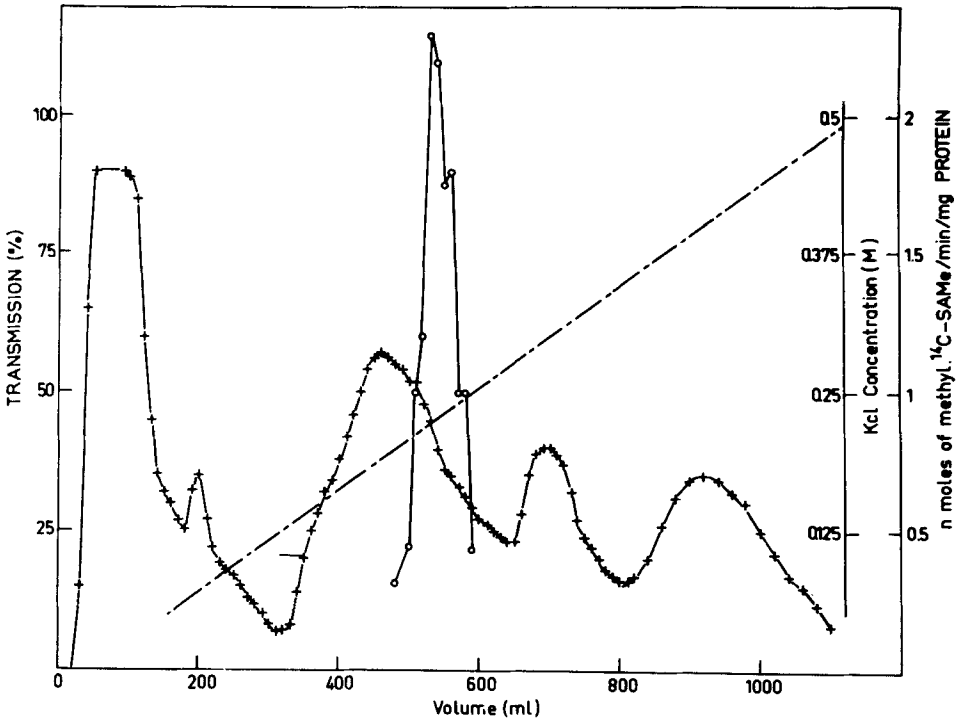

Fig.1 - Chromatography on DEAE cellulose of methionine-adenosyltransferase.

Desalted fraction is applied on the column (3.5 x 25 cm) of DEAE cellulose equilibrated with Tris buffer 50 mM pH 7.8, KCl 50 mM, $MgCl_2$ 5 mM, 2-mercaptoethanol 5 mM, glycerol 20 %. Elution with a linear gradient at KCl 50 mM to 500 mM in the same buffer.

x —— x transmission at 256 nm.
o —— o specific activity of enzyme (MAT).
. - . - KCl concentration.

2.2 - Preparation of L-[methyl-^{11}C] methionine

L-[methyl-^{11}C] methionine was synthetized by alkylation of homocysteine with [^{11}C] methyl iodide by a method described by COMAR et al. / 14 /. Three steps were required.

First $^{11}CO_2$ was prepared by the nuclear reaction ^{14}N (p,α) ^{11}C taking place in a N_2-O_2 mixture (95 % . 5 % v/v) irradiated by 16.5 MeV protons produced by a Cyclotron build by CGR (France).

Second, $^{11}CO_2$ was reduced to ^{11}C-methanol by $LiAlH_4$ in tetrahytrofurane, and transformed to ^{11}C-methyl iodide by hydriodic acid, the ^{11}C-methyl-iodide in transfered in to 200 µl acetone.

Third, L-[^{11}C-methyl] methionine synthesis resulted from the reaction of the ^{11}C methyl iodide with L-homocysteine. For this purpose to the 200 µl of the acetonic solution of ^{11}C-methyl-iodide were added 150 nmmoles of L-homocysteine thiolactone (chlorhydrate salt) in 150 µl water and 0.7 mmoles sodium hydroxyde in 70 µl water. The reaction vessel was tightly closed and heated at 50°. After 3 minutes, the solution was neutralized by HCl, and heating was continued for 4 minutes. Unreacted ^{11}C-methyl-iodide was removed by bubbling N_2 into the solution, while heating at 40°, 50-100 mCi of [^{11}C-methyl] methionine were thus routinely prepared.

2.3 - Preparation and purification of ^{11}C-methyl adenosyl-methionine

CANTONI / 7 / has described S-adenosyl-methionine synthesis by enzymatic condensation of L-methionine with ATP ; CANTONI's procedure has been adapted to the handling of [^{11}C-methyl] L-methionine as follows : in a total volume of 0.6 ml, were mixed : 30 µmoles, pH 8.5 Tris, 80 µmoles KCl, 50 µmoles $MgCl_2$; 0.5 µmole dithiotreitol, 10 µmoles ATP (disodium salt),

30-200 nmoles [^{11}C-methyl] L-methionine (specific radioactivity 100-1 000 Ci/mmoles), 540 µg of purified methionine-adenosine transferase, and incubated for 10 minutes at 37°C.

The whole incubation mixtures was poured on a twin packed column of 0.8 cm diameter, made of 9 cm of Biogel P-10. superposed into 2 cm AGIX8-HCO_3^-, and eluted with water.

In the upper gel, the proteins and S-adenosylmethionine were excluded, whereas ATP and methionine were slowed down and retained by theresin, fig. 2 (panel A).

The effluent of the mixed column contained S-adenosylmethionine as single labeled compound to control the purity of [^{11}C-methyl] S-adenosylmethionine, and aliquot (20 µl, 160 µCi) was injected onto Partisil SCX Whatman column (0.46 x 25 cm) and eluted by 0.4 M K H_2PO_4, pH 4.3 at a flow rate 2 ml/minute. The effluent was monitored by absorption at 254 nm and by ^{11}C scanning with an ionisation chamber. Figure 2 (panel B) shows the elution of a single UV absorbing and ^{11}C labeled compound. The shift between the two tracings of figure 2 (panel B) is due to the arrangement of the monitoring devices.

The mixed column was calibrated with [^{14}C-methyl] S-adenosylmethionine obtained by the same procedure and shown to yield a quantitative recovery of S-adenosylmethionine in 10 minutes. Thus, altogether synthesis and purification of S-adenosylmethionine could be carried out in 20 minutes with a final yield of 80 % computed on the methionine input. The specific radioactivity of the [^{11}C-methyl] S-adenosylmethionine obtained was 198 Ci/mmole and was measured 45 minutes after the end of N_2 bombardment.

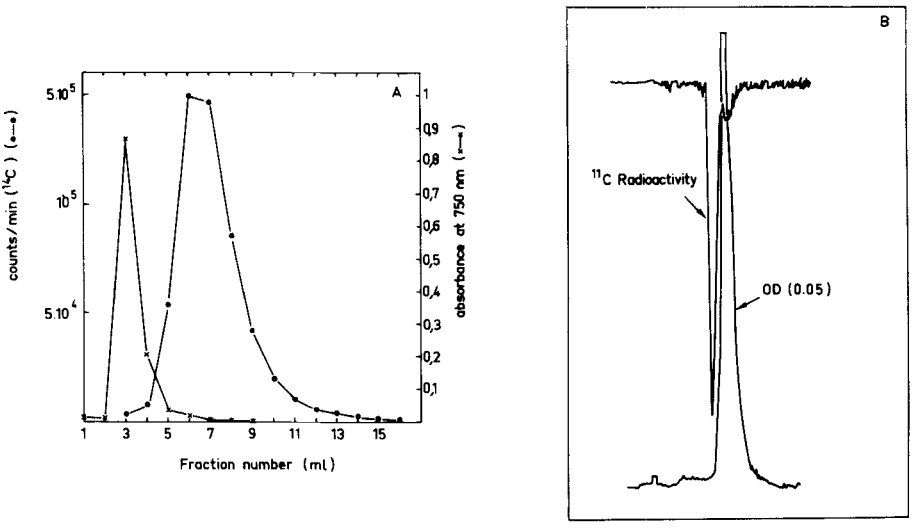

Fig.2 - Panel A : Purification of S-adenosylmethionine.
Water elution pattern of a mixture of ATP, methionine, S-adenosyl-methionine and methionine adenosyltransferase on a twin-packing column (Biogel P-10 : 0.8 x 9 cm and AG1X8 HCO_3^- whereas enzymes (x —— x) and ^{14}C-methyl S-adenosylmethionine (. —— .) where excluded.

Panel B : ^{11}C-radioactivity scanning and UV detection of labelled S-adenosylmethionine synthetized. Identification of the synthetized product was accomplished by comparing its retention time with a known reference compound and by co-injection with the reference compound.

3 - RESULTS AND DISCUSSION

Preliminary assays to prepare S-adenosylmethionine were performed by application of the published procedure / 8 , 10 / using ^{14}C-methionine to provide quantification. We had no difficulties in reproducing the data already described. For instance, following CAILLARD's protocole / 9 / we obtained a condensation of methionine with ATP of 22 % in 10 minutes and of 74 % in 60 minutes. However these results were not satisfactory since the first figure is too small and the second too time consuming when considering practical ^{11}C handling.

Furthermore the purification procedures had also to be shortened. To remain in the frame imposed by the ^{11}C half-life, the methionine-adenosyl-transferase was purified and the various parameters of the reaction were studied. The procedure described above was optimalized with respect to enzyme and ATP concentrations. Figure 3, panels A and B indicated that in the presence of 30 nmoles methionine, the rates of S-adenosylmethionine synthesis reached maximum values where 0.5 mg enzyme and 10 µmoles ATP were added. Under these conditions the conversion of methionine into S-adenosylmethionine reached a maximum also in 10 minutes. Figure 3, panel C showed that from the 30 nmoles methionine added, 80 % were converted into the required product.

The second improvement proposed concerned the use of twin-layers column, which in a single run, allowed the recovery of S-adenosyl-methionine deprived of free methionine and of proteins in a time no longer than 10 minutes. The twin-layer column is particularly suited to remote-handling manipulation for its simplicity, a prerequisite for routine operations.

The [^{11}C-methyl] S-adenosyl-methionine has been used in conjunction with methyl-transferases to prepare ^{11}C-methoxy-tyramine and [^{11}C-methyl] adrenaline for clinical investigations. It has also been directly introduced in a rabbit, and its repartition followed as a function of time by an opticamera CGR. 6 mCi of ^{11}C-S-adenosyl-methionine were found to label essentially

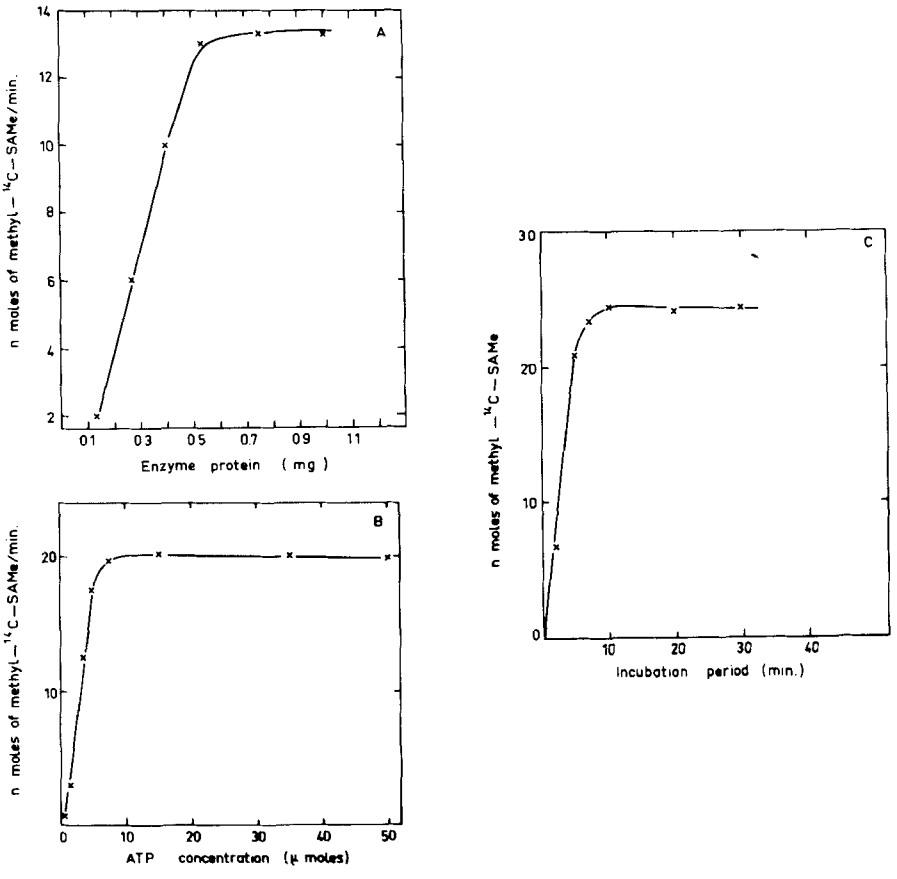

Fig. 3 - Effect of varying parameters on the enzymatic synthesis of S-adenosylmethionine.

Reactional mixture incubated 10 minutes at 37°C contains in a total volume of 0.6 ml : 30 μmoles Tris pH 8.5, 80 μmoles KCl, 50 μmoles $MgCl_2$, 0.5 μmoles dithiotreitol, 0.03 μmoles ^{14}C-methionine, 50 mCi/mmoles, 7 μmoles ATP. Panel A : in these above conditions the effect of varying enzyme concentration on the synthesis of S-adenosyl-methionine was studied between 0.1 mg and 1.1 mg. 0.540 mg represents the optimal value of methionine-adenosyltransferase (24 u/mg of enzymes). Panel B : Effect of various amount of ATP on the synthesis of S-adenosyl-methionine with 0.540 mg of enzyme in the same conditions described above the optimal value of ATP is found at 10 μmoles. Panel C : Effect of incubation period on the synthesis of S-adenosyl methionine with 10 μmoles ATP and 0.540 mg of enzymes. Results show an optimal incubation period of 10 minutes (final conditions are described in Chapter 2-3).

the kidneys with no important crossing of the blood brain barrier. No other specific localisation of radioactivity was observed Fig.4. These results are essentially similar to those obtained by EAKINS / 15 / after injection of ^{75}Se-adenosyl-methionine to the rat, despite the difference in label.

This similarity, followed in the present experiment, during 20 minutes reflected the multiplicity and the sizes of the adenosyl-methionine pools. The absence of ^{11}C differential methyl-concentrations might also express the general occurence of methyl transfer process occuring at comparable rates in the various tissues.

It might be anticipated that rapidly deviding cells should present a rate of absorption of [^{11}C-methyl]-adenosyl-methionine and fixation of the ^{11}C-methyl group higher than that of the ground tissues. More assays are needed to establish the reality of that point and its usefulness for clinical purposes.

At the present stage the main application of [^{11}C-methyl] adenosyl-methionine remains in vitro enzymatic labelling of metabolites. By analogy we are developing labelling on polypeptides or proteins by the use of methyltransferases specific for arginine lysine and γ-carboxyl group respectively / 16 /. This line of research might lead to a general process for intermove labelling of fragile macromolecules.

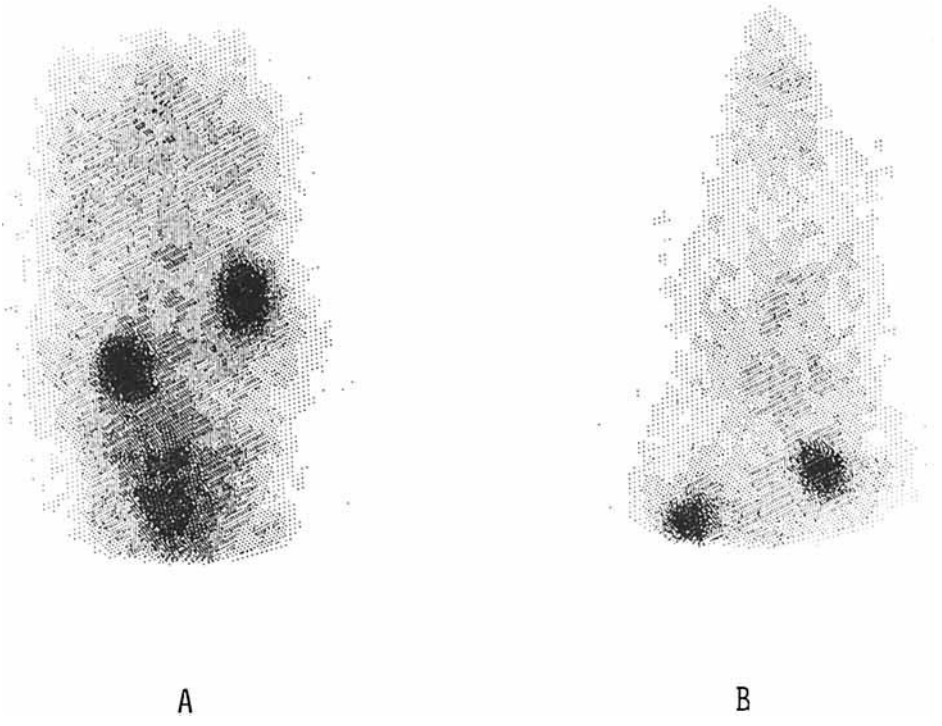

Fig.4 - The distribution of ^{11}C-methyl-S-adenosyl-methionine was followed during 24 minutes in a male rabbit after intravenous administration of 6 mCi of the labelled product. The radioactivity was detected externally by an opticamera CGR.

Imaging A : obtained just after injection from data accumulated during 1 minute shows a very high uptake of the radioactivity in the kidneys.

Imaging B : obtained during 1 minute (12 minutes after injection) also shows a high uptake of the radioactivity in the kidneys and some light radioactivity evenly distributed in the body. In order to verify the uptake of the radioactivity samples of brain and kidneys has been taken at the end of the experimentation after the death of the animal and the radioactivity has been measured in a NaI well counter. Results expressed in percentage of injected dose corroborate quite well the above observation (right kidneys 6.3 %, left kidney 4.9 %, brain 0.035 %).

REFERENCES

1 - WOLF, A. and REDVANLY, C.
Int. J. Appl. Rad. Isotopes (1977), Vol. 28, 29-48.

2 - COMAR, D., MAZIERE, M., BERGER, G., CROUZEL, C., SOUSSALINE, F. and KELLERSHOHN, C.
J. Fr. Biophys. and Med. Nucl. (1979), Vol. 3, n° 5, p. 285.

3 - MAZIERE, M., COMAR, D., MARAZANO, C. and BERGER, C.
Eur. J. Nucl. Med., 1, 255-258 (1976).

4 - BERGER, G., MAZIERE, M., KNIPPER, R., PRENANT, C. and COMAR, D.
Int. J. Appl. Radiat. Isotopes, 30, 393-399 (1979).

5 - REIFFERS, S., BEERLING-VAN DER MOLEN, H.D., VAALBURG, W., TEN HOEVE, W., PAANS, A.M.J., KORF, J., WOLDRING, M.G. and WYNBERG, H.
Int. J. Appl. Radiat. Isotopes, 28, 955-958 (1977).

6 - VAALBURG, W., BEERLING-WAN VER MOLEN, H.D., REIFFERS, S., RIJSKAMP, A., WOLDRING, M.G. and WYNBERG, H.
Int. J. Appl. Radiat. Isotopes, 27, 153-157 (1976).

7 - CANTONI, G.L.
J. Biol. Chem., 204, 403-416 (1953).

8 - CANTONI, G.L. and DURELL, J.
J. Biol. Chem., 225, 1033-1048 (1957).

9 - CAILLARD, L.
Thèse présentée à l'Université René Descartes,
Paris (1971).

10 - MATTHYSSE, S., BALDESSARINI, R.I. and VOGT, M.
Anal. Biochem., 48, 410-421 (1972).

11 - SCHLENK, F. and DE PALMA, R.E.
J. Biol. Chem., 229, 1051 (1957).

12 - LOMBARDINI and al.
Mol. Pharmacol., 6, 481-499 (1970).

13 - FOO PAN and TARVER, H.
Arch. Biochem. Biophys., 119, 429-434 (1967).

14 - COMAR, D., CARTRON, J.C., MAZIERE, M. and MARAZANO, C.
Eur. J. Nucl. Med., 1, 11-14 (1976).

15 - EAKINS, M.N.
Eur. J. Nucl. Med., 4, 101-103 (1979).

16 - SANGOUK KIM and CHOH HAOLI
Int. J. Peptide Protein Res. 13, 282-286 (1979)