ENZYMATIC SYNTHESIS OF ^{11}C-LABELLED (-)-EPINEPHRINE

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SUMMARY

An enzymatic method is described for the synthesis of ^{11}C-labelled (-)-epinephrine in which the [^{11}C]methyl group is sequencially transferred from L-[methyl-^{11}C]methionine to (-)-norepinephrine via S-adenosyl-L-[methyl-^{11}C]methionine respectively by the enzymes L-methionine-S-adenosine transferase (M.A.T) and phenylethanolamine-N-methyl transferase (P.N.M.T). Chromatographically pure ^{11}C-epinephrine having a specific activity approximately 200 Ci/mmol is obtained 35 minutes after the synthesis of the starting compound L-[methyl-^{11}C]methionine.

Preliminary results are presented for the dynamics of the distribution of the ^{11}C-epinephrine in organs of the rabbit by gamma scintigraphy.

- Key words : (-)-epinephrine, enzymatic synthesis, ^{11}C-labelling, scintigraphy.

INTRODUCTION

(-)-Epinephrine is a molecule acting essentially as an hormone-neurotransmitter (BORCHARDT[1]) and secreted mainly by adrenals. It does not cross the blood-brain-barrier, but a small amount is synthetized in some brain areas (SAAVEDRA[2], MILBY et al.[3], KOPP et al.[4], WEIL-MALHERBE et al.[5]).

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^{11}C-Labelled epinephrine was synthetized in order to visualise in vivo the binding sites of epinephrine by external detection and to estimate the effects of varying physiological and pharmacological parameters on their function. An enzymatic synthesis was selected because of poor yields in the small scale chemical synthesis from [^{11}C]methyl iodide or [^{11}C]formaldehyde for example, and the short half-life of ^{11}C (20.4 minutes). The method consists in methylating (-)-norepinephrine, a precursor of (-)-epinephrine, by using a methyl donor, L-[methyl-^{11}C]methionine and two enzymes : L-methionine-S-adenosine transferase (M.A.T.)(E.C. 2.4.2.13) and phenylethanolamine-N-methyltransferase (P.N.M.T.) (E.C. 2.1.1.28).

The two steps of the reaction are schematized as follows :

$$\text{ATP} + \text{L(-)}\ {}^{*}\text{CH}_3\text{-methionine} \xrightleftharpoons{\text{M.A.T.}} {}^{*}\text{CH}_3 \sim \text{S.A.M.} + \text{pyrophosphate} + \text{phosphate}$$

$$\text{(-)-norepinephrine} + {}^{*}\text{CH}_3 \sim \text{S.A.M.} \xrightleftharpoons{\text{P.N.M.T.}} \text{(-)}\ {}^{*}\text{CH}_3\text{-epinephrine} + \text{S.A.H.}$$

(S.A.M. : S-adenosylmethionine ; S.A.H. : S-adenosylhomocysteine)

The first step of this process uses a well established synthesis to enzymatically convert L-[methyl-^{11}C]methionine and ATP forming S-adenosyl-L-[methyl-^{11}C]methionine (GUEGUEN et al.[6]).

In contrast with chemical synthetic methods, enzymatic methylation of norepinephrine results in minimal amounts of the by product N-methylepinephrine. Furthermore the methyl donor, L-[methyl-^{11}C]methionine can be ^{11}C-labelled by an automated process (BERGER et al.[7]) ; then levogyre S-adenosyl-L-methionine is enzymatically obtained and acts immediately as coenzyme in the second step. These coupled reactions results in both time saving of synthesis and avoids decomposition of S-adenosylmethionine.

The aim of our work (SOUSSAIN[8]) was to prepare the enzyme which catalyses biosynthesis of epinephrine, optimalize the whole enzymatic process in order to obtain a high yield in a short time, then isolate and analyse radiolabelled epinephrine before injecting it in an animal.

MATERIALS

ATP was purchased from Boehringer Manheim (GFR) ; S-adenosylmethionine chloride was obtained from I.C.N. (USA), dithiothreitol from Calbiochem (USA), (-)-norepinephrine and (-)-epinephrine from Sigma and Merck and L-methionine from Merck (GFR).

Ion exchange resin Biorex 70 (100-200 mesh) was from Biorad (USA) and gel permeation Sephadex G 50 medium from Pharmacia.

L-[methyl-^{14}C]Methionine and (-)-S-adenosyl-L-[methyl-^{14}C]methionine were prepared by Commissariat à l'Energie Atomique (France). L-[methyl-^{3}H]Methionine was purchased from New England Nuclear Corporation (USA).

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

L-Methionine-S-adenosine transferase, extracted from rat liver by a method adapted from LOMBARDINI et al.[9], was kindly supplied by Choay Institute (Paris).

Bovine adrenal glands were provided by C.N.R.Z. Jouy-en-Josas, Essonne (France).

The cells rupture chamber was from Kontes (USA).

Fluorimetric analysis of norepinephrine and epinephrine was achieved with a Farrand-Foci-Mark I spectrofluorometer.

High pressure liquid chromatography was performed on pre-packed cation exchange Partisil-10-SCX Whatman (25 x 0.46 cm) and C_{18}-phase Radial Pak A Waters (10 x 0.8 cm).

Precoated silicagel plates (10 x 20 cm) for thin-layer chromatography (H.P.T.L.C. Kieselgel 60) were obtained from Merck.

Ultraviolet light, iodine and autoradiography were used for visualization.

Autoradiography was achieved on RP-O mat medical X-ray films (Kodak).

Radioscans of TLC plates were carried out with a Berthold Scanner II.

Tritium and carbon-14 determinations were made with a SL 30 Intertechnique liquid scintillation counter. Gamma radioactivity was measured with solid scintillation counters.

METHODS

Enzymatic purification of phenylethanolamine-N-methyl-transferase : P.N.M.T.

P.N.M.T. converts norepinephrine into epinephrine through N-methylation. This enzyme was extracted from adrenal medulla where endogenous plasma epinephrine is synthetized ; catecholamines are also stored there (IVERSEN[10]) ; so endogenous epinephrine has to be eliminated from the enzymatic preparation in order to avoid P.N.M.T. activity being inhibited ; such epinephrine also induces unwanted isotopic dilution.

Elimination of catecholamines during each step of enzyme purification, was followed by fluorometric analysis based on oxidation of the amines to their fluorescent trihydroxyindole derivatives : noradrenolutine and adrenolutine, with ascorbic acid in an alkaline solution as stabilizing reagent, according to the procedure of QUEK et al.[11].

The method of extraction was based on studies concerning the enzyme (AXELROD[12], CONNET et al.[13], POHORECKY et al.[14], DELARUE[15]). A high purity was not needed for our purpose, but inhibitory effect or by-reaction had to be avoided ; so we built up a procedure as simple as possible while checking every stage as follows : fluorimetric analysis of catecholamines, determination of protein utilizing the principle of protein dye binding (BRADFORD[16]), assays for P.N.M.T. activity and C.O.M.T. activity. In order to test presence of C.O.M.T., the enzymatic preparation was incubated with [methyl-^{14}C]SAM, catecholamine and $MgCl_2$; then methoxylated catecholamine which could have been synthetized was extracted with n-butanol from an aqueous solution buffered with 0.2 M borate pH 9.5 (partition coefficient of metanephrine : 0.69 ; partition coefficient of epinephrine : 0.02). No C.O.M.T. activity was detected.

Bovine adrenal glands (25 g) were dissected to remove cortice part and the adrenal medullas were homogenized in 2 volumes of 0.05 M phosphate buffer (pH 7.5). The suspension underwent nitrogen pressure (150 bars during 10 minutes) followed by sudden expansion in a cell rupture chamber in order to release intracellular enzymes. After centrifugation of the homogenate at 27,000 g for 20 min., then 100,000 g for 90 min., the proteins in the supernatant were fractionated with 4 M ammonium sulfate solution ; the fraction precipitating between 1.2 M and 2 M was dissolved in a small volume of phosphate buffer pH 7.5 (5 ml).

Epinephrine and norepinephrine were simultaneously estimated by differential fluorometric analysis based on fluorescence readings at 2 sets of wavelengths - activating/fluorescent wavelengths - one at 400 nm/505 nm another one at 415/515 nm ; amounts were 0.025 and 0.006 micromole per mg of protein respectively.

The precipitate obtained at isoelectric point after dialysis against 5×10^{-2} M acetate buffer (pH 5) for several hours, was collected by centrifugation and removed ; an aliquot of supernatant containing 50 mg protein was applied to a Sephadex column G 50 medium (50 x 1.7 cm) and eluted with 10^{-2} M ammonium acetate solution at a flow rate of 0.2 ml per min. Epinephrine and norepinephrine were thus removed.

Elution profile : Figure 1.

The eluted fractions containing significant quantities of enzyme activity were pooled and lyophilized. The enzymatic preparation was stored at - 40°C for several months without loss of activity.

Prior to use, a sample of the enzymatic preparation was dissolved (20 mg per ml) in a protective mixture (POHORECKY et al.[14]) containing 10^{-2} M phosphate buffer pH 7.5 - glycerol (90-10, v/v) and 10^{-3} M dithiothreitol to prevent oxidation of thiol groups which are required for enzyme activity (CONNETT et al.[13]).

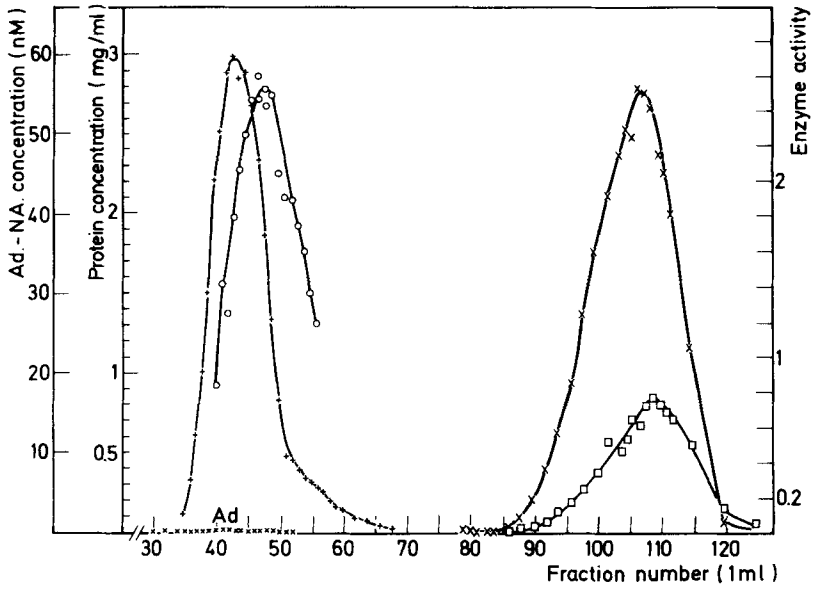


Fig. 1 : P.N.M.T. purification on Sephadex G 50 : elution profiles ; 1 ml fractions were collected. + ——+ : protein concentration ($mg.ml^{-1}$).

o——o : enzyme activity : expressed as nanomoles of substrate transformed during 15 min.

x——x : epinephrine (Ad.) concentration (nM).

□——□ : norepinephrine (NA.) concentration (nM).

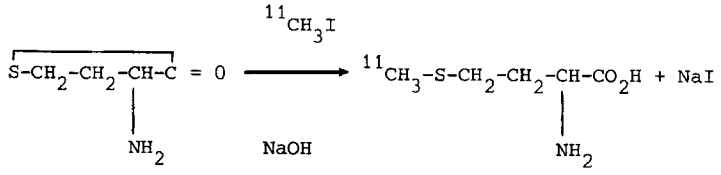
Preparation and purification of (-)[methyl-^{11}C]epinephrine

L-[methyl-^{11}C] Methionine which is the methyl donor in enzymatic reaction was obtained by methylation of L-homocysteine with [^{11}C]methyl iodide (BERGER et al.[7]).

The precursor $^{11}CH_3I$ was prepared from $^{11}CO_2$ which was formed by nuclear reaction $^{14}N(p,\alpha)^{11}C$, taking place in a nitrogen target containing traces of oxygen under pressure, irradiated for 30 min. at intensity 30 μA, by 20 MeV protons produced with a cyclotron (CGR-France).

$^{11}CO_2$ was reduced by $LiAlH_4$ in tetrahydrofuran to [^{11}C]methanol, then transformed into [^{11}C]methyl iodide by hydriodic acid while heating and the $^{11}CH_3I$ trapped in 200 µl acetone.

L-[methyl-^{11}C]Methionine was synthetized from $^{11}CH_3I$ in acetone and L-homocystein thiolactone (1 µ.mole in 75 µl water), in a basic solution (36 µ.moles NaOH) (BERGER et al.[7]).



When the reaction ended, acetone was removed by heating in a nitrogen stream ; excess of sodium hydroxide was neutralized by 20 µ.moles hydrochloric acid (4 µl of 5 N HCl) then 250 to 300 µl water were added while rinsing out inner surfaces of glass tube to dissolve the L-[methyl-^{11}C]methionine which was thus prepared in 28-31 min.

Optimal conditions for the two stages of the enzymatic synthesis of the ^{11}C-epinephrine were developed taking into account the small quantity of L-[methyl-^{11}C]methionine (ca. 50 n.moles) and the necessity for short reaction times (less than 15 minutes). The second step catalysed by P.N.M.T. was studied and supported by investigations on P.N.M.T. (AXELROD[12], DELARUE[15], PARVEZ et al.[17], GRIPOIS et al.[18], CONNETT et al.[13,19]), while the first step enzymatic synthesis of S-adenosyl-L-methionine was supported by the previous studies (GUEGUEN et al.[6]). The following reaction mixture gave a high transmethylation yield : pH 8.7 Tris-HCl buffer : 150 µmoles ; dithiothreitol : 0.5 µmole ; ascorbic acid : 0.5 µmole ; $MgCl_2$: 25 µmoles ; KCl : 150 µmoles ; A.T.P. : 5 µmoles ; norepinephrine : 0.1 µmole ; P.N.M.T. preparation : 5.7 mg ; purified M.A.T. : 0.65 mg, in a total volume of : 700 µl. The neutralized solution containing 45 to 50 n.moles of L-[methyl-^{11}C]methionine was introduced with a syringe in the reaction tube and incubated for 12 minutes at 40°C ; the proteins were then precipitated by 110 µl of 1.25 M trichloroacetic acid ; the

precipitate was removed by filtration under pressure with a syringe and the filtrate collected in a vial containing 70 µmoles of sodium hydroxide to neutralize excess of trichloroacetic acid.

Isolation of ^{11}C-epinephrine in a small volume, from other labelled molecules which could alter interpretation of images, was performed on H.P.L.C. cation exchange column ; the mobile phase consisted of 5 x 10^{-3} M citrate buffer pH : 5.4 (70 per 100 trisodium citrate, 30 per 100 citric acid), 5 x 10^{-4} M sodium chloride ; this solution could be injected in the animal ; the flow rate was 2 ml/min and the absorbance measured at 280 nm ; 1 ml fractions eluate were collected and radioactivity counted in a solid scintillation detector in order to assess and determine the most ^{11}C-epinephrine concentrated fractions (Fig. 2).

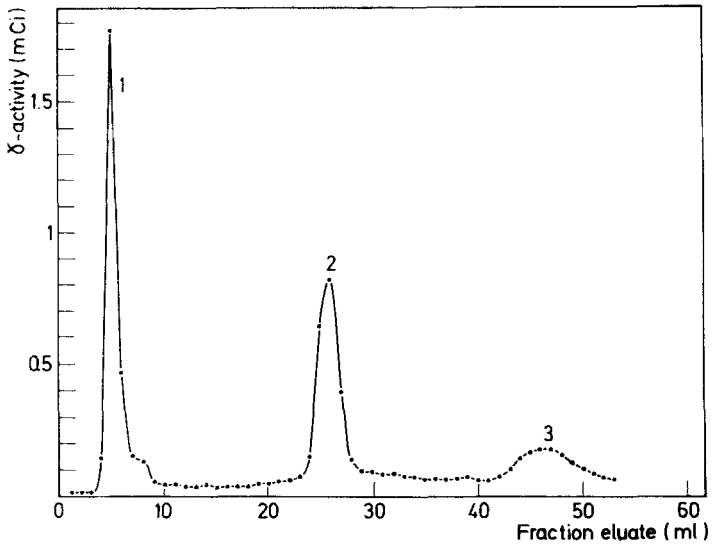


Fig. 2 : Purification of ^{11}C-epinephrine : high performance cation exchange liquid chromatography on Partisil-SCX, after protein removal ; mobile phase : 5 x 10^{-3} M citrate buffer, pH 5.4, 5 x 10^{-4} M sodium chloride ; flow rate 2 ml/mn ; the radioactivity counted in a NaI detector was corrected for background and physical decay.

Peak 1 : ^{11}C-methionine. Peak 2 : ^{11}C-epinephrine.

Peak 3 : S-adenosyl-L-[methyl-^{11}C]methionine.

According to the evaluation, the operations by remote control such as : transfer of ^{11}C-methionine into incubation mixture, precipitation of proteins, filtration, injection onto the chromatographic column, involved loss estimated at about 40 per cent. Thus, synthesis and purification of ^{11}C-epinephrine from ^{11}C-methionine could be carried out in 30-35 min with a final yield of 20 % from ^{11}C-methionine. The quantity of purified ^{11}C-epinephrine obtained in 3 ml of eluent buffer was of 1.5 mCi, this radioactivity being measured 65 minutes after the end of bombardment.

The identity, purity and specific activity of ^{11}C-epinephrine obtained were checked by liquid chromatography on C-18 phase radial-pak column ; the mobile phase was 2 x 10^{-2} M phosphate buffer pH 2.3 with 3 per 100 methanol (v/v) ; the elution was carried out at flow rate 2 ml/min and the effluent monitored by U.V. absorption at 254 nm and by radioactivity scanning with an ionisation chamber (Fig. 3).

The product obtained was chromatographically pure ; according to radioactivity monitoring which is very sensitive, ^{11}C-epinephrine was free of other radiocompounds ; its specific radioactivity was 170 Ci/mmol and was measured 72 minutes after the end of N_2 bombardment.

RESULTS AND DISCUSSION

Carbon-14 and tritium labelled substrates were used to optimise the enzymatic reaction kinetics with respect to (i) the quantity of L-[methyl-^{11}C]methionine (ca.50 n.moles) and (ii) the decay of ^{11}C.

In order to estimate simultaneous activity of methionine-S-adenosine transferase (M.A.T.) and phenylethanolamine-N-methyltransferase (P.N.M.T.), the following three labelled compounds had to be separated at the end of incubation time : unused methionine, intermediate methyl donor S-adenosyl-L-methionine and epinephrine. The separation was achieved by stepwise elution from short columns (8 cm x 0.42 cm^2) of weak cation-exchange resin : Bio Rex 70 (100-200 mesh). At the end of enzymatic reaction, the whole incubation mixture was applied onto a

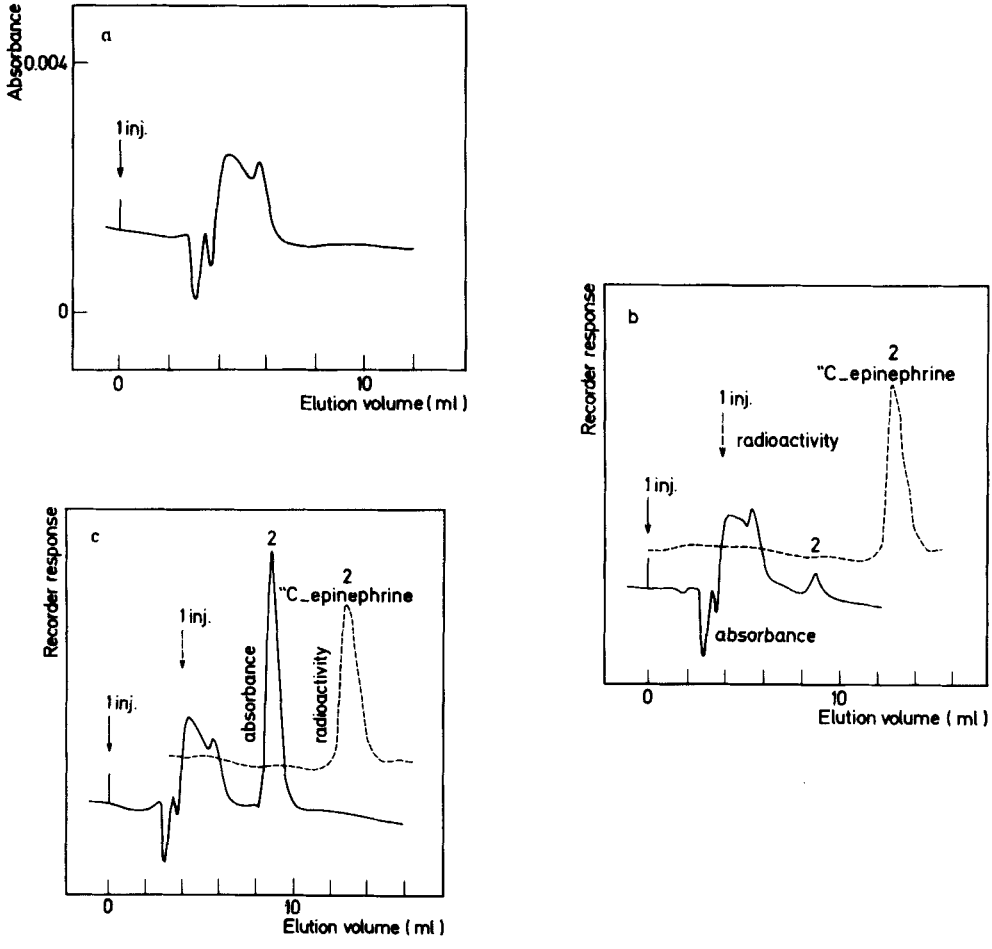


Fig. 3 : Chromatographic assay of ^{11}C-epinephrine : identity, chemical and radiochemical purity.

Stationary phase : C 18 radial pak.

Mobile phase : 0.02 M phosphate buffer pH 2.3 - methanol 3 p 100 (v/v).

—— : UV absorption curve 254 nm, 0.01 AUFS.

--- : radioactivity curve. 1 : injection. 2 : epinephrine elution peak.

The shift between the two tracings is due to arrangement of the monitoring devices. Panel a : injection of citrate buffer only. 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: Panels b and c.

column of resin which had previously been equilibrated with 0.01 M sodium acetate buffer pH 6 ; methionine was eluted with 12 ml of the same buffer, then epinephrine was eluted with 16 ml of 0.4 M acetic acid containing 5×10^{-3} M $MgCl_2$ and S-adenosylmethionine was finally eluted with 12 ml of 5 M acetic acid containing 5×10^{-3} M $MgCl_2$ (Mg^{II} lowered the distribution coefficients of epinephrine and S-adenosylmethionine). To determine these labelled compounds, an aliquot (1 ml) of each of the three effluent fractions was mixed with 10 ml of scintillation solution and counted. The results were expressed as quantity of labelled epinephrine synthetized under given conditions such as incubation time and amount of enzyme protein in the reaction mixture.

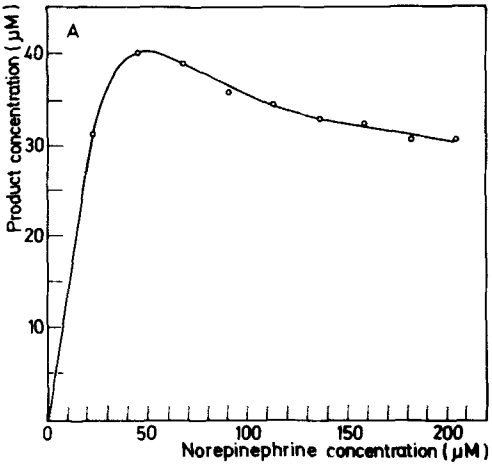
In order to obtain an adequate quantity of radiolabelled epinephrine within minimum time, the conditions of P.N.M.T. reaction were optimised, then those of M.A.T. activity (GUEGUEN et al.[6]) were taken into account in optimising the whole reaction.

Although no ion is required for P.N.M.T. activity (CONNETT et al.[13]), the inhibitory effect of the substrate norepinephrine on P.N.M.T. activity at the necessary high substrate concentrations (50-100 µM ; Fig. 4), was reduced by increasing the ionic strength of the incubation mixture with KCl (0.16 M) (CUBEDDU et al.[20]). Potassium ion is necessary for M.A.T. activity and the overall effect was to increase the yield in the enzymatic reaction by 25 per cent.

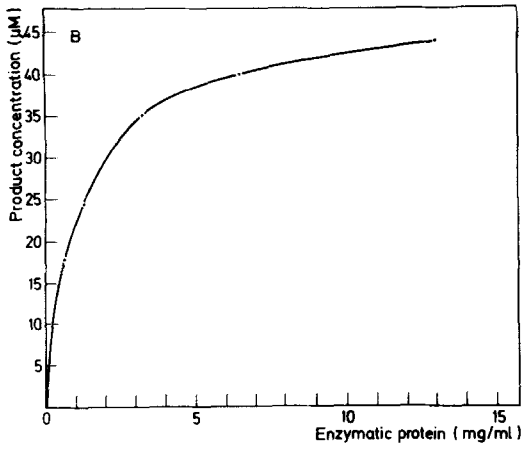
Study of other parameters indicated the use of 5-6 mg of enzyme protein (Fig. 4) and to buffer the incubation mixture for simultaneous M.A.T. and P.N.M.T. activities with Tris/HCl pH 8.5-9 ; magnesium was required for M.A.T. activity, but was inhibitory at high concentration ; 2.5×10^{-2} M $MgCl_2$ was added. According to the kinetics of enzymatic methylation at 40°C (Fig. 4) optimum incubation time for ^{11}C-labelling was reached in 10-15 min.

Analysis of enzymatic synthesis showed a side reaction of P.N.M.T. which catalyses methylation of epinephrine to N-methylepinephrine (AXELROD[21]) with a very small yield. Identification of enzymatic ^{14}C-labelled N-methylepinephrine

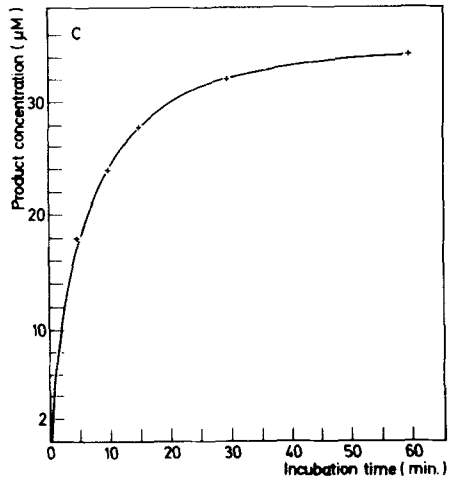
Fig. 4 : Effect of varying parameters on the enzymatic synthesis of epinephrine.



Panel A : norepinephrine concentration effect on P.N.M.T. activity before adjusting ionic strength ; reaction mixture incubated 15 min. at 37°C contained : 0.2 M Tris-HCl buffer pH 8.5, 10^{-3} M dithiothreitol, 10^{-4} M S-adenosyl-L-[methyl-^{14}C]-methionine (specific activity 1.25 mCi/mmol) 10 mg/ml P.N.M.T. protein and increasing concentration of norepinephrine.



Panel B : effect of P.N.M.T. fraction concentration on synthesis of epinephrine after optimalization of other parameters : incubation time was 10 minutes at 40°C ; ionic strength was increased with 0.1 M NaCl ; norepinephrine concentration was 60 μM; 6 mg/ml enzyme preparation was found to be convenient.



Panel C : methylation kinetics of norepinephrine with methionine as substrate, M.A.T. and P.N.M.T. as enzymes. Reaction mixture incubated at 40°C contained : 0.16 M Tris-HCl buffer pH 8.5, 2.5×10^{-2} M $MgCl_2$, 0.16 M KCl, 5.4 mM A.T.P., 55 μM norepinephrine, 45 μM ^{14}C-methionine, 5.5 mg/ml P.N.M.T. preparation, 0.5 mg/ml M.A.T. preparation.
Results show that the resultant transmethylation rate significantly decreased after 10 min. incubation time.

was carried out by silicagel thin layer chromatography (solvent system : n-butanol-acetic acid-water 33 : 8.5 : 8.5, V/V/V) and cation exchange H.P.L.C. ; the reference non-labelled compound was obtained by reductive methylation of norepinephrine or epinephrine (15 µmoles) using formaldehyde (60 µmoles) and sodium cyanoborohydride (19 µmoles) in acetonitrile (3 ml) as solvent with acetic acid (30 µl) as proton donor, according to the method of BORCH used for preparation of tertiary methylated amines (BORCH et al.[22,23], BERGER et al.[7]).

The amount of the by-product of enzymatic synthesis, N-methylepinephrine, became significant only after an incubation time higher than 20 minutes (Fig.5).

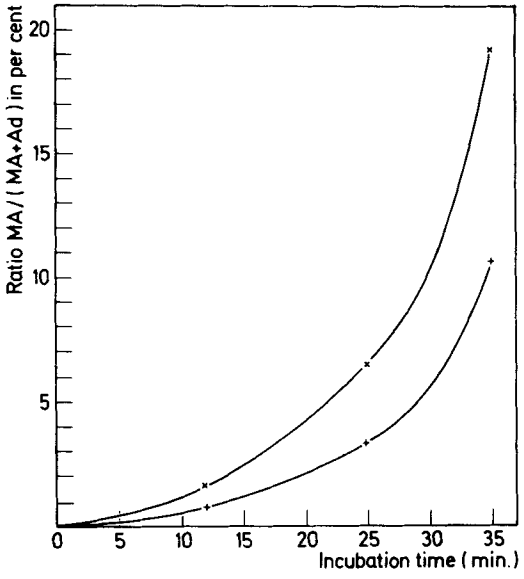


Fig. 5 : Enzymatic synthesis of methylepinephrine (M.A.) ; as a proportion of the total product of enzyme activity (epinephrine + methylepinephrine), as a function of incubation time. Substrate is norepinephrine.

x—x : ratio expressed on account of radioactivity
(2 labelled methyl groups per molecule of methylepinephrine).

+—+ : ratio expressed on account of molecular amount.

Incubation temperature : 41°C.

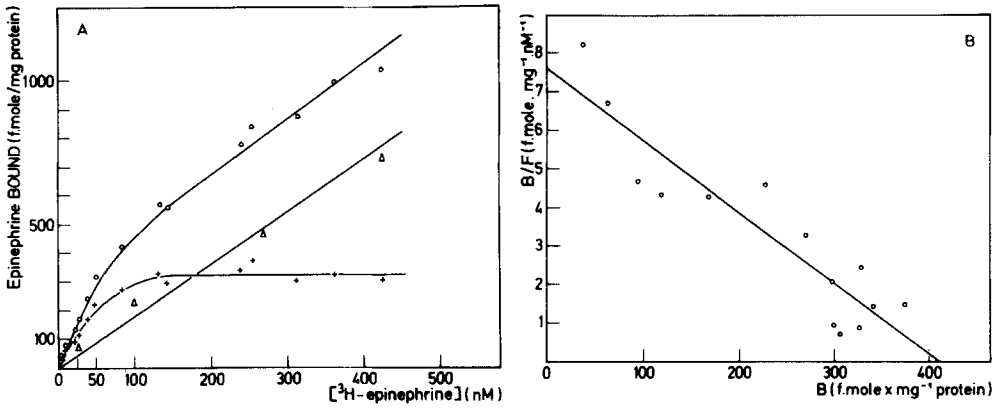


Fig. 6 : Binding of ^{3}H-epinephrine to rat liver plasma membrane as a function of ^{3}H-epinephrine concentration. Specific activity of ^{3}H-epinephrine : 50 Ci/mmol.

Panel A : Rat liver plasma membranes (700 µg in a final volume of 400 µl) were incubated with constant shaking at 25°C for 30 min. with the indicated concentrations of ^{3}H-epinephrine in 50 mM Tris/HCl buffer, pH 7.5 containing 10 mM $MgCl_2$, 10^{-5} M (-)propranolol as β-antagonist, 8 x 10^{-4} M ascorbic acid, 3 x 10^{-3} M catechol, 2.5 x 10^{-5} M iproniazide. Binding was studied in the absence of α-antagonist : phentolamine (total binding o——o) and in the presence of 10^{-5} M phentolamine (non saturable binding Δ——Δ) (saturable binding +——+ calculated as the difference between total and non saturable binding). Bound fractions were isolated by filtering through Whatman GF/C glass fiber and washing with 15 ml of incubation buffer at 4°C then counted in scintillation vials after adding 1 ml methanol and 10 ml scintillation mixture. Each value was the mean of triplicate determinations.

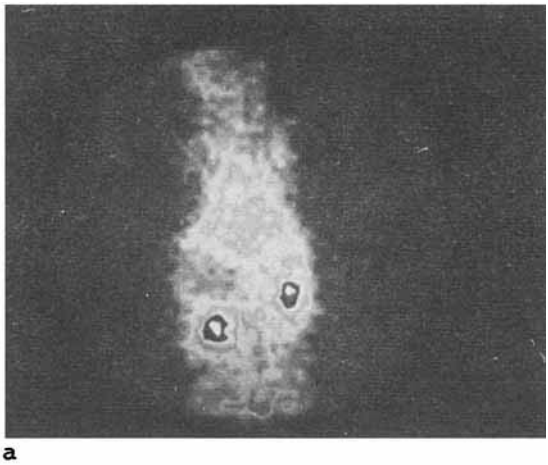
Panel B : Scatchard plot : the ratio B/F of bound ^{3}H-epinephrine f.mole/mg protein to free ^{3}H-epinephrine (nM) is plotted as a function of bound ^{3}H-epinephrine (f.mole/mg) (correlation coefficient : r = 0.92) ; dissociation constant calculated from the slope : K_D = 54 nM ; B_{max} = 412 f.moles/mg (Hill coefficient was n_H = 0.91).

The characteristic parameters of the hormone receptor binding were checked through a saturation experiment of α-adrenergic receptors of the hepatic plasma membranes which were prepared from albino, Wistar rats according to the procedure devised by Neville (GUELLAEN et al.[24]), using pure tritiated epinephrine at high specific activity (50 Ci/mmol) prepared from L-[methyl-^{3}H]methionine as substrate in the enzymatic synthesis.

The capacity of specific binding sites (410 f.moles/mg of membrane protein at 25°C) and the affinity of epinephrine (dissociation constant : K_D = 54 nM) determined by Scatchard analysis (Fig. 6), were comparable with the results obtained with (-)-^{3}H-norepinephrine under the same experimental conditions : number of sites = 340 ± 70 f.moles/mg membrane protein ; K_D = 130 ± 60 nM (P. GEYNET[25]).

The affinity test for the α sites offered the advantages of differentiating the two enantiomers since agonist binding is stereospecific : the affinity of (+) epinephrine for the α-adrenergic receptor is 12-fold lower than that of the (-) epinephrine (GUELLAEN et al.[24]).

Purified [methyl-^{11}C]epinephrine prepared according to the method described above was collected in 2 or 2.5 ml of citrate buffer after H.P.L.C. ; the solution was then sterilized by passage through 0.22μm Millipore filter and injected intravenously in a living rabbit (3 kg) which had been anesthetized (levomeprazine I.M. : 12 mg ; pentobarbital I.V. : 30 mg) and placed in a ventral position. At time : 72 minutes after end of cyclotron run, ^{11}C-epinephrine (220 μCi) was injected into the ear vein, then tissue distribution of the labelled compound was explored using a scintillation camera (Anger Camera) and blood kinetics were followed by counting blood samples. The whole animal could not be centred, so consequently, the brain was not investigated because epinephrine and its major metabolite (metanephrine) (KOPIN et al.[26]) do not cross blood brain barrier (WEIL-MALHERBE et al.[5], PEYRAUD et al.[27]). The images of radioactivity integrated at various times (Fig. 7) indicated distribution was mainly in heart, liver-pancreas, kidneys, suprarenal glands, then in bladder



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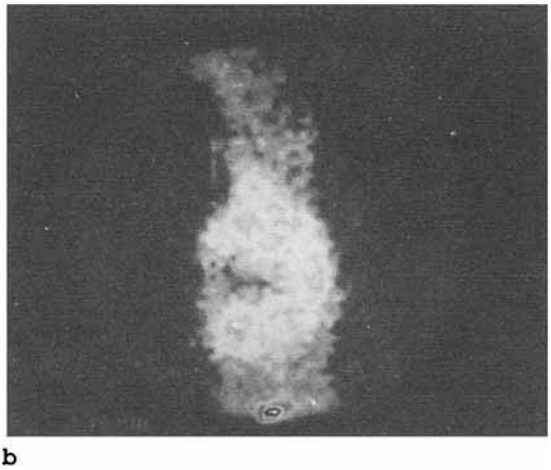

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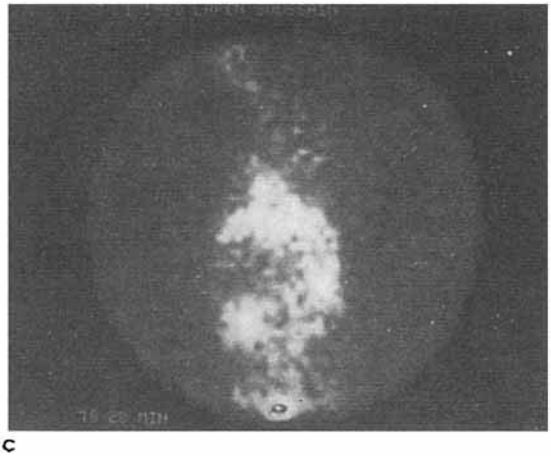

c

Fig. 7 : Thorax and abdomen distribution at different times after I.V. injection (time 0) of ^{11}C-epinephrine observed by gamma-camera in anaesthetized rabbit.

Acquisition of data from :

- time 0 to 8 min : Panel a.
- time 8 to 18 min: Panel b.
- time 18 to 28 min : Panel c.

The images achieved after computer process showed relative radioactivity distribution.

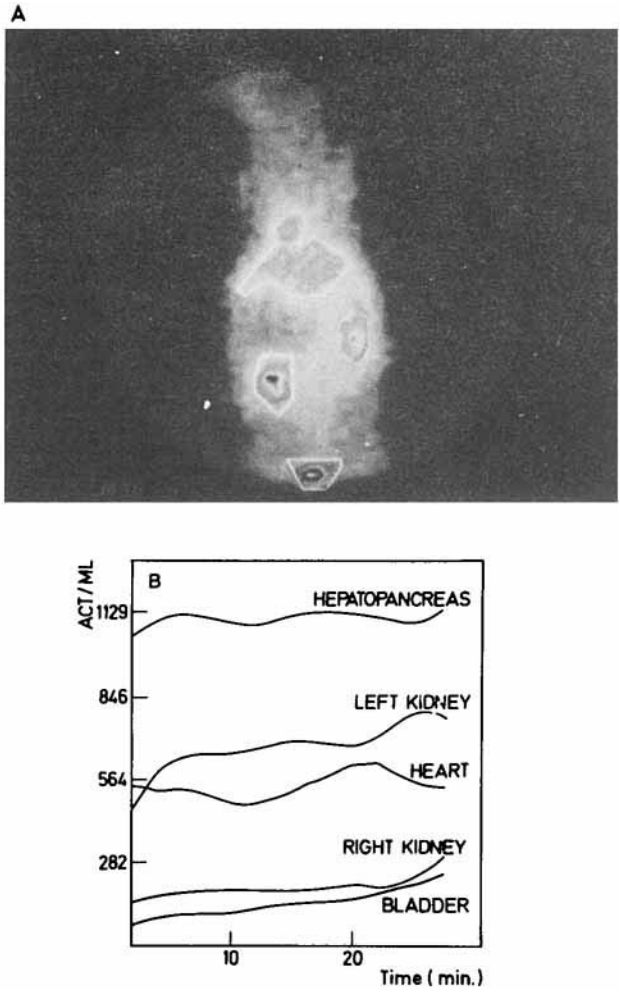

Fig. 8 : Dynamics of distribution of ^{11}C-epinephrine (or its major labelled metabolite) in various organs of the rabbit. For acquisition of data, selected polynomial areas visualized on Panel A were counted and the counts corrected back to the injection time.

Panel B : changes in ^{11}C concentration (radioactivity per ml of tissue in arbitrary unit) in selected areas .

after about ten minutes ; likely spleen was also labelled ; the lack of labelling in the place of lungs should be noted. It should be observed that the images depended on the binding sites of various organs, metabolism of labelled compound and position of organs which were visualized.

According to dynamic time studies (Fig. 8), concentration of ^{11}C-containing molecules remained approximately constant during the 28 minutes observation in liver-pancreas and heart ; it increased in kidneys (by about 40 per cent in left kidney and 100 per cent in right one, which is near the liver considering the resolution of camera) and in bladder : the radioactivity (corrected for physical decay of ^{11}C) was there, about 3.5 fold higher at the end of observation. This evolution of labelling could be compared to the elimination function of these organs. According to WIELAND et al.[28] uptake process should occur in adrenal glands.

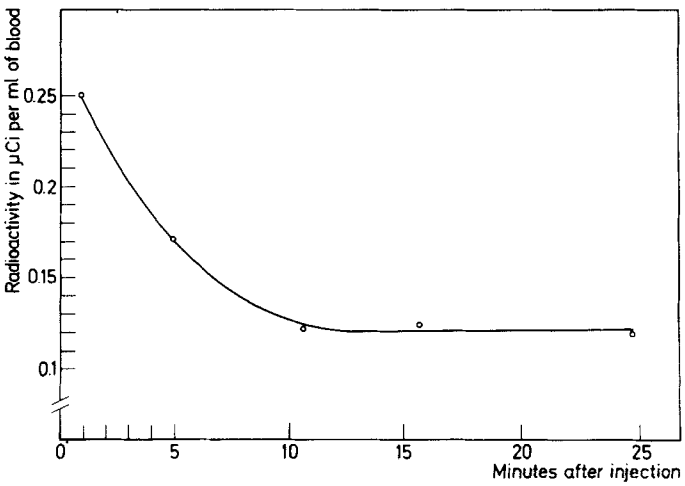

Fig. 9 : Blood kinetics of radiocompound in anaesthetized rabbit, after injection of 220 µCi ^{11}C-epinephrine. Blood samples were collected from ear vein. The radioactivity has been measured in a NaI well counter calibrated with Ge 68 standard ; the counts were corrected for background then for physical decay during acquisition time and corrected back to the middle injection time ; the blood quantity was then taken into account. The equation of regression curve was calculated : $y = 171.48\ e^{-t/4.41} + 115.72$ (ordinates unit : nCi/ml) ; and global biologic half-life of the rapid process deduced : $T_{1/2} = 3$ min.

According to the blood kinetic curve, resulting from the disappearence of epinephrine and the release of labelled metabolites in the circulating blood, a value of 3 minutes was obtained for the global biologic half-life of the rapid process (Fig. 9).

Some parameters may be modified during pathological processes and more assays are required to establish the usefulness of this molecule for clinical purposes, possibly by using the positron camera to extend the observations. The influence of injected drugs upon tissue distribution and displacement kinetics of ^{11}C-epinephrine might also lead to further studies.

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