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THE "IN VIVO" DISTRIBUTION OF CARBON 11 LABELED (—)-NICOTINE IN ANIMALS. A METHOD SUITABLE FOR USE IN MAN

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During the last twenty years, several authors have shown some interesting biological properties of nicotine especially its very rapid traverse of the blood-brain barrier (1) and its high uptake in many organs such as lungs, kidneys, adrenals, brain, hypophysis and the retina (2,3). Its cerebral metabolism is also very fast (4).

With the eventual aim of medical diagnosis in man, various properties have been verified "in vivo" in animals.

Carbon 11 was chosen to label nicotine for the following reasons :

1 - its "carrier free" preparation enables ^{11}C to be obtained with a very high specific activity (10^4 curies/μMole theoretically).

2 - its short half-life (20.4 minutes) permits injection into man without giving too high an absorbed dose .

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3 - its mode of disintegration being by emitting positrons which on annihilation give two 511 KeV gamma rays emitted at 180° allows both external detection by a gamma camera and a transaxial tomographic reconstruction with a positron camera.

4 - Finally as ^{11}C is a radioisotope of carbon 12 its incorporation into a molecule does not change the chemical and the physiological properties of that molecule.

Unfortunately, in view of the short half-life of ^{11}C, the labeling must be very fast and the yield of each step of the synthesis must be as high as possible. Also on account of the high toxicity of nicotine, it was necessary to obtain a labeled product with a very high specific activity in order to inject minute amounts of nicotine with a radioactivity high enough to allow an "in vivo" study of the product lasting one hour.

In addition, the injectable ^{11}C nicotine must be chemically and radiochemically pure, apyrogenic and sterile.

METHOD

The ^{11}C-Nicotine labelling was performed by methylation of nor(—)-nicotine (5) with ^{11}C-formaldehyde according to the BORCH reaction (6) instead of the ESCHWEILER CLARKE (7) and MEANS and FEENEY (8) methods which we described earlier (9).

The schema of the synthesis can be divided into four steps :

1 - $^{11}CO_2$ Production

^{11}C is produced by irradiation of nitrogen in a cyclotron according to the $^{14}N\ (p\alpha)\ ^{11}C$ nuclear reaction. As there are some traces of oxygen in the target wall and target gas we obtain directly $^{11}CO_2$ at the output of the target, (figure I).

2 - ^{11}C-Formaldehyde Synthesis

The $^{11}CO_2$ is reduced by $AlLiH_4$ into ^{11}C-methanol which is dehydrogenated into ^{11}C-formaldehyde by a silver catalyst in the presence of oxygen at 500°C (10).

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3 - Nor-Nicotine Methylation

The reaction of ^{11}C-formaldehyde with nor-nicotine gives an imine which is reduced by sodium cyanoborohydride into ^{11}C-nicotine (figure II).

4 - Purification

The chemical and radiochemical purifications are performed by high pressure liquid chromatography on a silica column. The sterilization is made by filtration on millipore membrane.

MATERIALS

On account of the short half-life of ^{11}C quite high quantities of radioactivity were used during the synthesis in order to obtain about 20 mCi of ^{11}C-nicotine as it is generally necessary for an "in vivo" study.

As 1 mCi of carbon 11 delivers at contact 15 rem/Hour, we had to develop a semi-automatic method of synthesis. All the preparations are now carried out in a closed lead-shielded cell (figure III). There is no contact of the fingers with the vessels containing the radioactivity except with the syringe containing the ^{11}C-nicotine ready to inject (11).

The reaction vessels are 1 ml conical tubes joined together by flexible teflon capillaries tightly fitted to hypodermic needles. These tubes are closed with plastic septa (Carlo Erba) and the passage of fluid is controlled by electrovalves (Durrum). The mobile reaction vessels are handled by remote control and are easily transferred from a hot bath to a cold one, (figure IV).

EXPERIMENTALS

As shown in figure V, carbon 11 dioxide produced "carrier free" by the 20 MeV proton irradiation of nitrogen (Air Liquide N60) is trapped in a copper coil cooled to -183°C in liquid oxygen.

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The $^{11}CO_2$ released by heating the coil (30°C) is carried by a current of nitrogen 2% oxygen into the first tube containing 50 µl Tetrahydrofuran + 5 µl $AlLiH_4$ 1,5 M.

During a subsequent heating, the solvent is evaporated and the complex $AlLi(OCH_3)_4$ is hydrolysed by 70 µl water.

The ^{11}C-methanol is then distilled and carried away by the current of nitrogen-oxygen into a 500 mg silver wool-filled quartz furnace (diameter : 4 mm ; length : 15 cm) kept at 500°C where it is dehydrogenated. A trap of Porapak P is placed at the input of the furnace in order to eliminate any trace of THF. The ^{11}C-formaldehyde obtained is transferred in the second tube containing the nor-nicotine (1 µM) solution in acetonitrile (260 µ l) with CH_3COOH (2 µl) - H_2O (70 µ l) and sodium cyanoborohydride in ethanol (1 µM). The reaction tube is closed by the electrovalves and heated at 50°C for 10 minutes. After this reaction time the mixture is injected automatically onto the chromatographic column and the tube is rinsed out with solvent fed in from outside by a syringe. (Waters chromatograph UV detector - column Magnum 9 partisil 50 cm Whatman).

Elution is carried out with 95/5(V.V.) mixture of chloroform and ethanol the latter containing 2.5 % water and 1.5 % ethylamine, at a flow rate of 8 ml/min.. The fraction corresponding to ^{11}C-nicotine is easily selected as shown in the chromatogram (figure VI) and evaporated under nitrogen current to dryness (after acidification pH2 because nicotine base is very volatile and taken up by physiological saline buffed to pH4.

After an automatic sterilization through a Millipore membrane (0.22 µM) we obtain directly in the syringe the ^{11}C-nicotine ready for injection. The overall time for the preparation of injectable ^{11}C-nicotine is about 35 minutes starting from $^{11}CO_2$ trapping. After a 45 minutes irradiation by 20 MeV protons at 20µ A beam current, nearly 100 mCi ^{11}C-nicotine can be prepared.

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The product is chemically and radiochemically pure, apyrogenic and sterile. The chemical yield from $^{11}CO_2$ is about 50 %. The specific radioactivity at the time of injection is around 500 mCi/µMole (400-700 mCi/µMole). This means that for 20 mCi of ^{11}C-nicotine injected in a living organism the mass of nicotine is quite small (5 µg).

ANIMAL STUDIES

The studies we have perform already on the ^{11}C-nicotine "in vivo" distribution in animals (9) have shown that after I.V. administration :

- in MICE, the lung elimination of $^{11}CO_2$ from the ^{11}C-nicotine demethylation is about 5-7 % of the injected radioactivity (100 µCi) during the hour following injection.

- in RABBITS, anesthetized with pentobarbital, the radioactivity following 2 mCi I.V. ^{11}C-nicotine, measured during one hour externally by a gamma camera (Nuclear Chicago pho gamma III fitted with a 511 KeV "high energy" parallel channel collimator and connected to a N M 40 "Informatek System") is, during the first minutes after injection, quite high in the head, lung, liver and kidneys of the animal (figure VII). The brain activity decreases sharply with time (figure VIII). The activity remains high in the liver and kidneys during the time of the examination.

In order to know more about the "in vivo" intracerebral distribution of ^{11}C-nicotine, we continued our study on monkeys (Baboon papio-papio) with a positron camera (ORTEC-ECAT) which is an emission computerized tomograph using annihilation coincidence detection to produce transaxial tomographic images of the distribution of positron emitting radionuclide.

The ^{11}C-nicotine distribution was generally studied during 40 minutes after administration of 20 mCi I.V. ^{11}C-nicotine.

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In order to follow the evolution of the radioactivity in the brain, the study is performed three times in four brain slices of 2 cm width and 1 cm displacement parallel with the inion-orbital line. This includes the total cerebral matter of the monkey.

During the examination, the animal anesthetized with ketamine is lying on the positron camera bed. The first brain transverse slice is centered on the inion-orbital line (OI) of the animal. Accurate positioning is facilitated by a laser beam dispersed in a plane corresponding to the centre of the 2 cm wide slice. The acquisition of a sequence of four adjacent slices is performed automatically under control of the computer. The basic duration of a slice is selected according to the count rate observed immediately after injection, the system then automatically increases the duration of subsequent slices to correct for decay of the isotope. Thus with ^{11}C the first slice might be requested for 120 seconds but the forth would actually take around 200 seconds.

The figure IX shows images of the first scan of sequence of four brain slices after I.V. administration in a monkey (male, weight : 17 Kg) of 23 mCi ^{11}C-nicotine. (R.A.S. 243 mCi/μM).

1 represents the slice centred on OI and 2 cm wide. Slices 2,3,4 are of 2 cm width and 1 cm apart. So the overall scan of the brain is 5 cm wide.

As we advance in the cortical region (image 4), the radioactivity becomes more homogenously distributed. The uptake is very high in the temporal lobe (right and left side of 1-2-3) in the cerebellum (2 bottom) and in the occipital and frontal lobe (3 up and down). In 4, we see the especially high uptake of the occipital cortex at the interhemispheric scissure.

The radioactivity in 1 between and beneath the temporal lobe may be localised in the pons and medulla oblongata.

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The figure X shows the evaluation of the radioactivity in the same brain slices during the second scanning sequence (i.e. 10 minutes after injection) : the radioactivity decreases and becomes less selective (2-3-4) on the other hand as the radioactivity in the eyes remains high. They become visible at the very top of the image 1.

In order to better demonstrate the less selective uptake of the radioactivity the three successive images obtained of the second brain slice are presented together in figure XI :

1 - 4.20 minutes after injection,
2 - 14 minutes " "
3 - 27.30 minutes " "

Also, to see if this rapid reduction in activity is the same in all parts of the brain, three zones in this slice have been selected as shown in figure XII :

1 - corresponds to a cerebral hemisphere slice,
2 - corresponds to the temporal region,
3 - corresponds to the eyes.

In these three zones, the radioactivity has been quantified at the three scan times (4, 14, 27 minutes). As seen from this figure, the activity decreases in the same way in both brain regions ; however, in the eyes, the dispersion is much less rapid (results are expressed in % injected dose per gramm of tissue $X10^{-2}$).

So as to consider a possible displacement of ^{11}C-nicotine by cold nicotine in some specific regions of the brain and to evaluate the permeability of the blood brain barrier to nicotine, an identical study was made one week later, in the same monkey but after a load of nicotine (I.V. 100 µg/Kg). As shown on figure XIII (first scan of the sequence) and on the curve of the figure XIV, the results are not significatively different. Unfortunately during the experiment, the head of the monkey slipped and so the slices are displaced one unit downwards with respect to those of the previous examination. Consequently slice 4 is almost out of the brain. The brain kinetics shown in the figure XV (which represents the first and the second scan

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of the second brain slice) show a decrease and a more uniform radioactivity distribution in the latter slice except for in the eyes. This result is identical to that of the previous experiment.

IN CONCLUSION, A method is described to label nicotine with carbon 11. A hundred millicuries can be obtained, in 45 minutes, with a high specific activity.

This labeling of nicotine has allowed an "in vivo" study of the distribution of this very toxic drug in animals.

Five minutes after injection in rabbits or monkeys, it was shown with a gamma camera or with a positron camera that the radioactivity was very rapidly distributed throughout the tissues especially in brain,lungs and kidneys.

^{11}C-nicotine readily penetrates the blood-brain barrier and the brain radioactivity decreases very sharply with time. The eyes however retained activity, possibly in the retina as has been demonstrated previously with ^{14}C-nicotine (12). Unfortunately the monkey is not the ideal subject for ^{11}C-nicotine brain study because :

- the brain is small, considering the resolution of the cameras and the cerebral lobes are also quite overlaped in this animal.

- Japanese authors (13) have shown that compared with dogs the nicotine brain uptake is lower, due to the high affinity of nicotine for skeletal muscle which occupies approximately forty to fifty % of the body weight of the monkey (14). Also in monkeys, the nicotine destruction is faster than in dogs because there is a higher enzyme nicotine metabolizing activity in the liver of this animal (15).

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The differences observed between various animals studies using nicotine indicate that we should not draw any firm conclusions about the behaviour of this drug in humans.

In order to do so, examinations must be conducted in man and the method described in spite of its limitations provides a means for such a study.

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LEGENDS

Figure I - ^{11}C-formaldehyde Synthesis.

Figure II - ^{11}C-nicotine scheme of the synthesis.

Figure III- Lead schielded cell : outside.

Figure IV - Lead schielded cell : inside.

Figure V - ^{11}C-nicotine synthesis scheme of the apparatus.

Figure VI - ^{11}C-nicotine H.P.L.C. chromatogram.

Figure VII- γ camera image of a rabbit after I.V. injection of 2 mCi of ^{11}C-nicotine.

Figure VIII - Brain radioactivity curve of a rabbit after I.V. injection of 2 mCi of ^{11}C-nicotine.

Figure IX - Positron camera image of the first scan of a sequence of four brain slices after I.V. administration, in a monkey, of 23 mCi ^{11}C-nicotine (R.A.S. 240 Ci/mMole)

Figure X - Positron camera images : Second scanning sequence of the same slices as shown in IX (10 minutes after injection).

Figure XI - Positron camera images of second brain slice rewed at different times after injection :

1 - 4,20 minutes after injection
2 - 14 " " "
3 - 27,30 " " "

Figure XII - ^{11}C-nicotine brain kinetics in different zones :

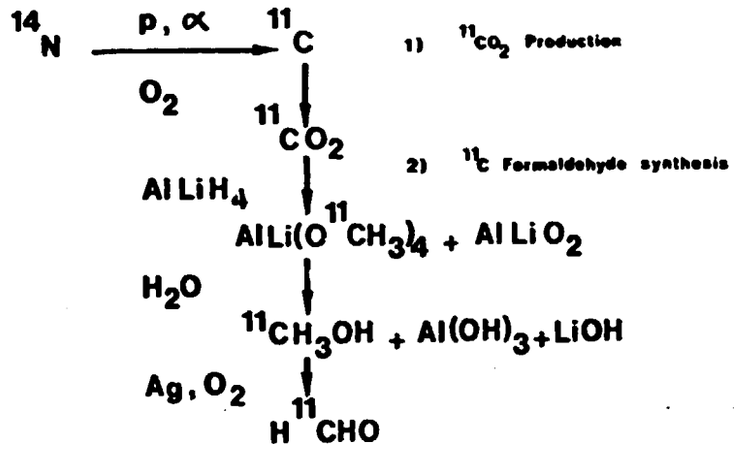
1 - corresponds to a cerebral hemisphere
2 - " " the temporal region
3 - " " the eyes

Figure XIII - Positron camera images : first scan of the sequence of four brain slices after I.V. administration in a monkey of 23 mCi ^{11}C-nicotine (R.A.S. 2,3 Ci/mMole).

Figure XIV - ^{11}C-nicotine brain kinetics in different zones after a load of nicotine (I.V. 100 µg/Kg) :

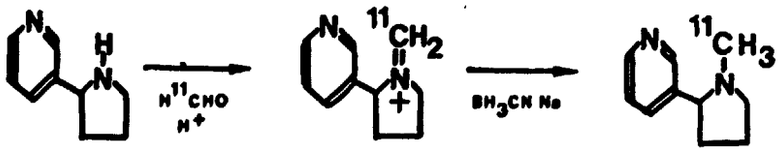
1 - corresponds to a cerebral hemisphere
2 - " " the temporal region
3 - " " the eyes

Figure XV - Positron camera images : first and second scan of the second brain slice after I.V. administration, in a monkey, of ^{11}C-nicotine (R.A.S. 2,3 Ci/mMole).



^{11}C Formaldehyde synthesis

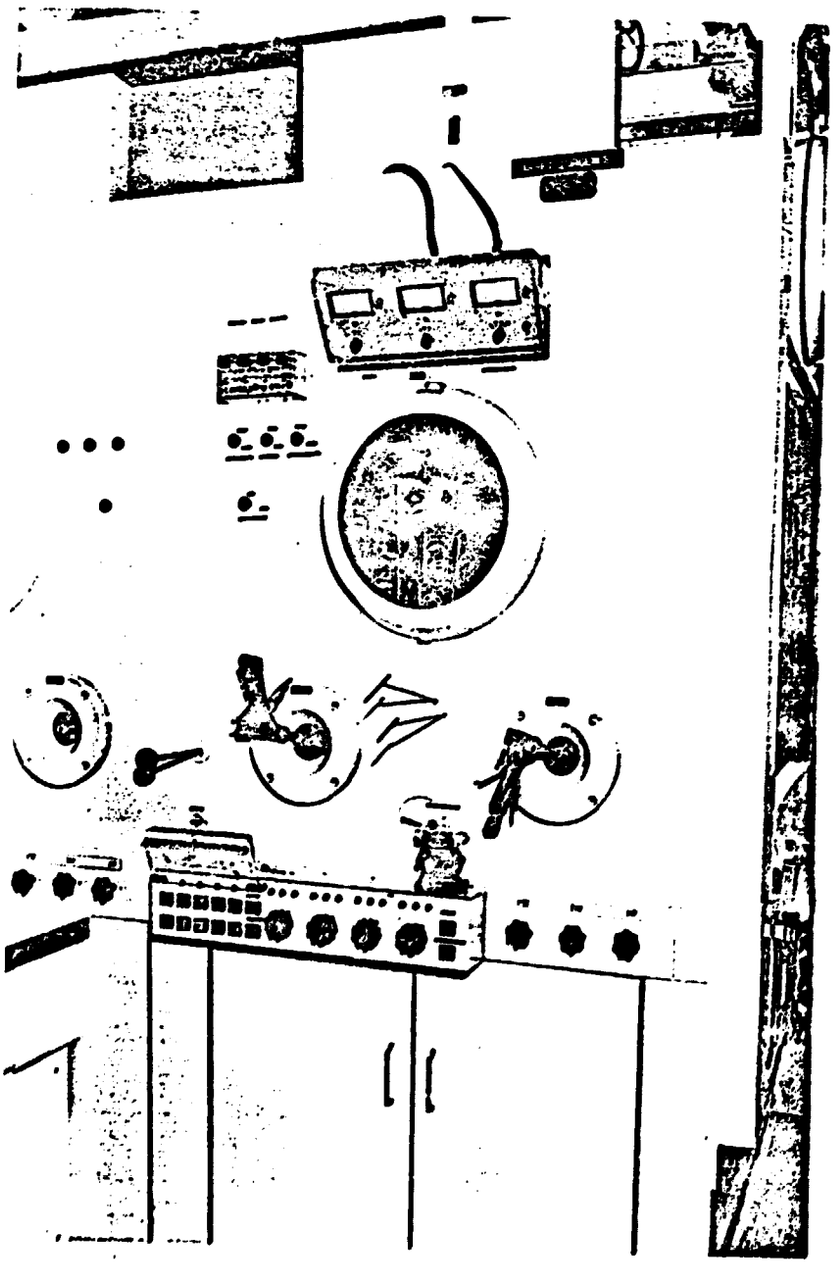
I

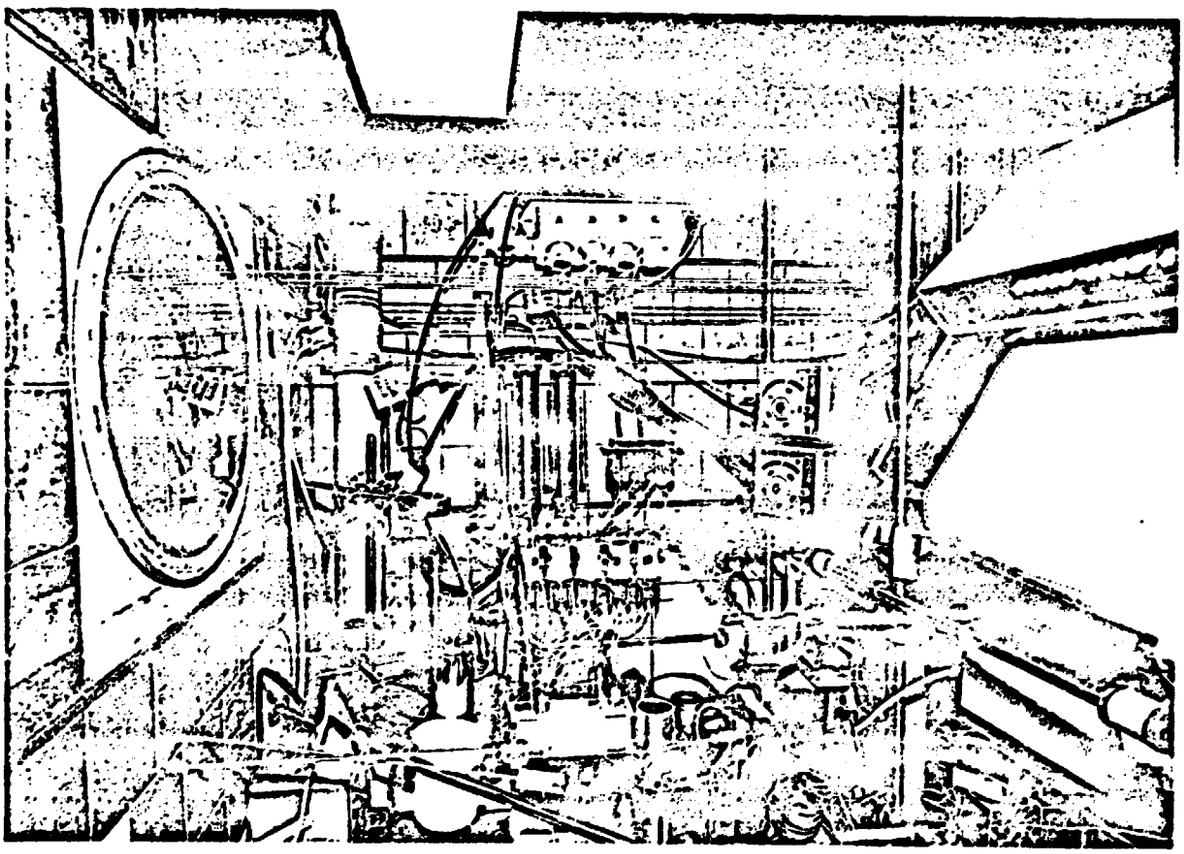


Nornicotine Imine ^{11}C Nicotine

3) ^{11}C Nicotine synthesis

II





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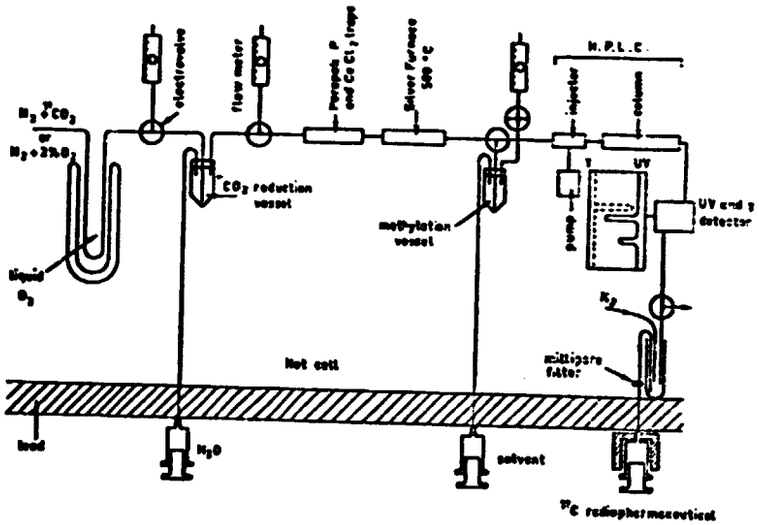
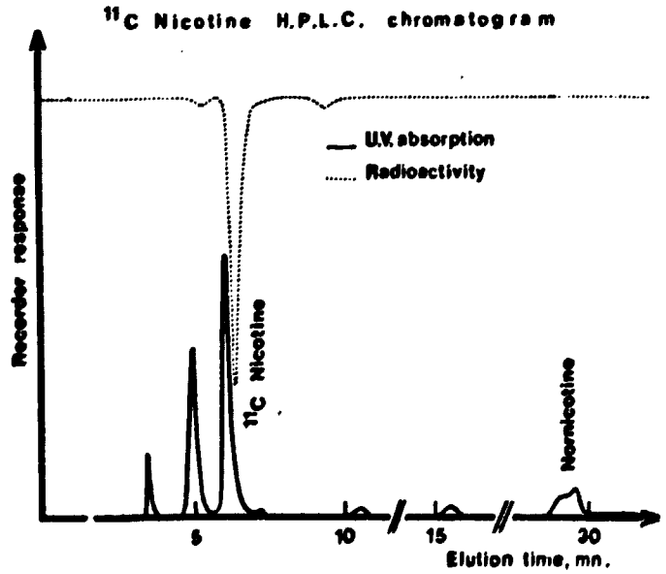
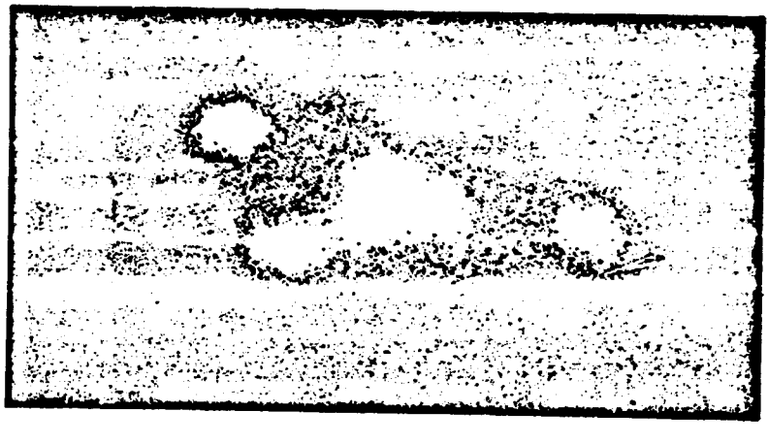

electrovalve
flow meter
Peroxide P and Cu Cl2 traps
Silver Furnace 500 °C
H.P.L.C.
injector
column
UV
pump
UV and γ detector
CO_2 reduction vessel
methylation vessel
liquid N_2
N_2
Hot cell
millipore filter
lead
H_2O
solvent
^{11}C radiopharmaceutical

V


^{11}C Nicotine H.P.L.C. chromatogram
U.V. absorption
Radioactivity
Recorder response
^{11}C Nicotine
Nornicotine
5
10
15
20
Elution time, mn.

VI



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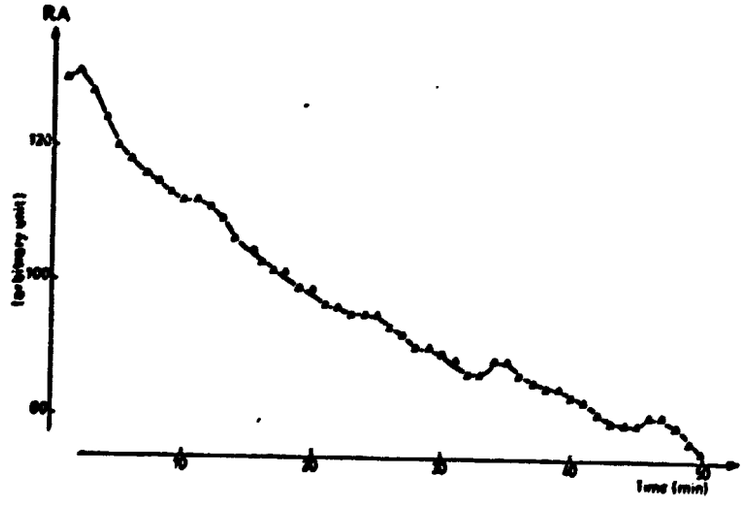

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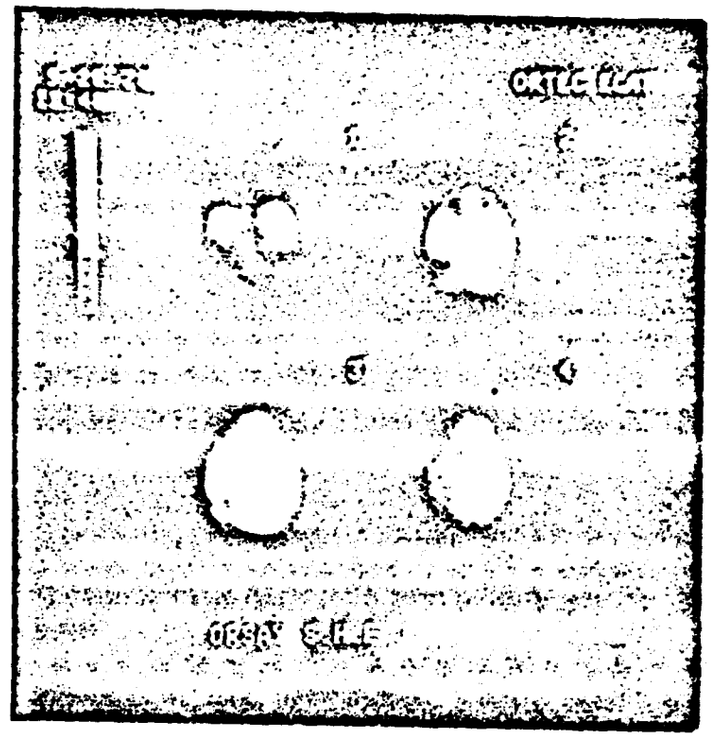

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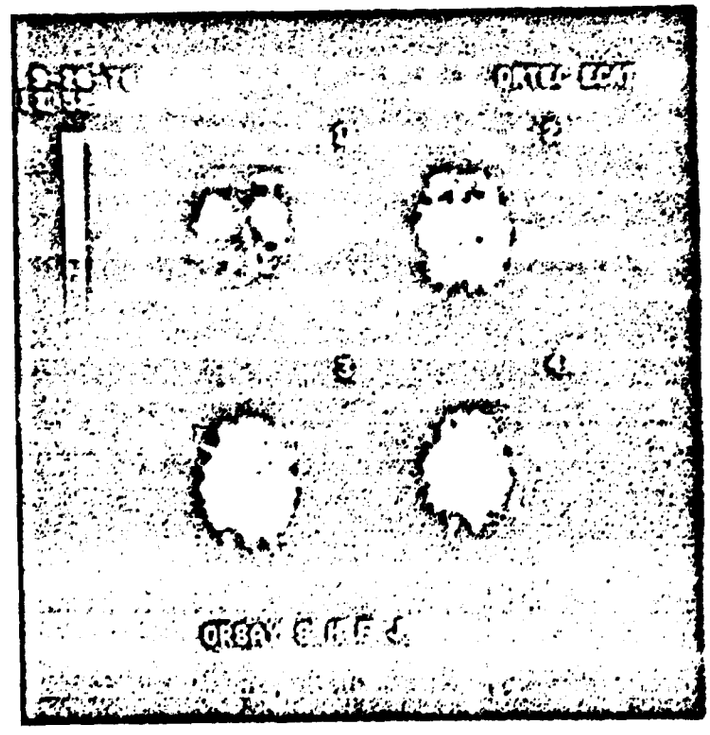

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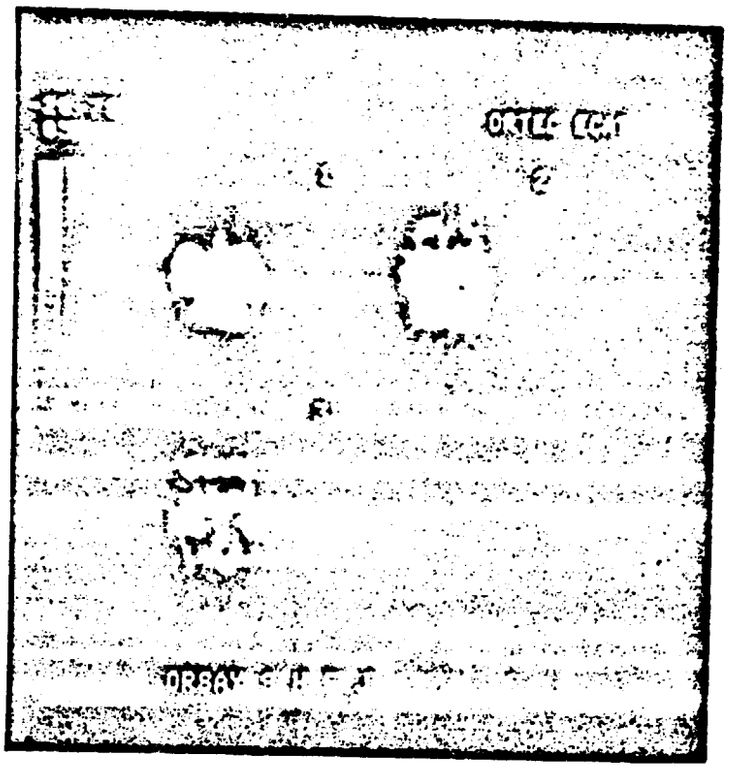

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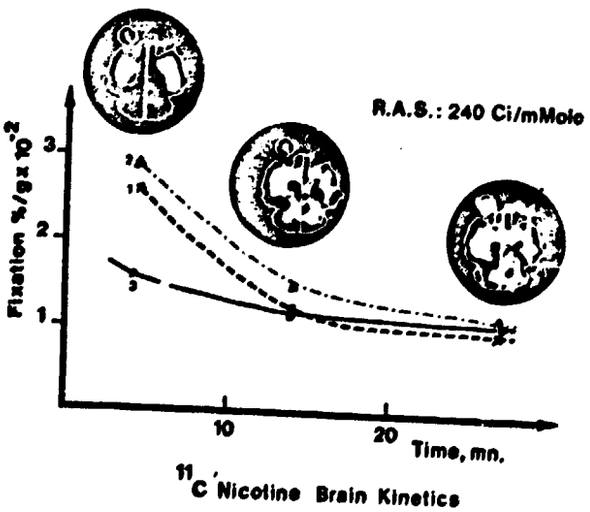
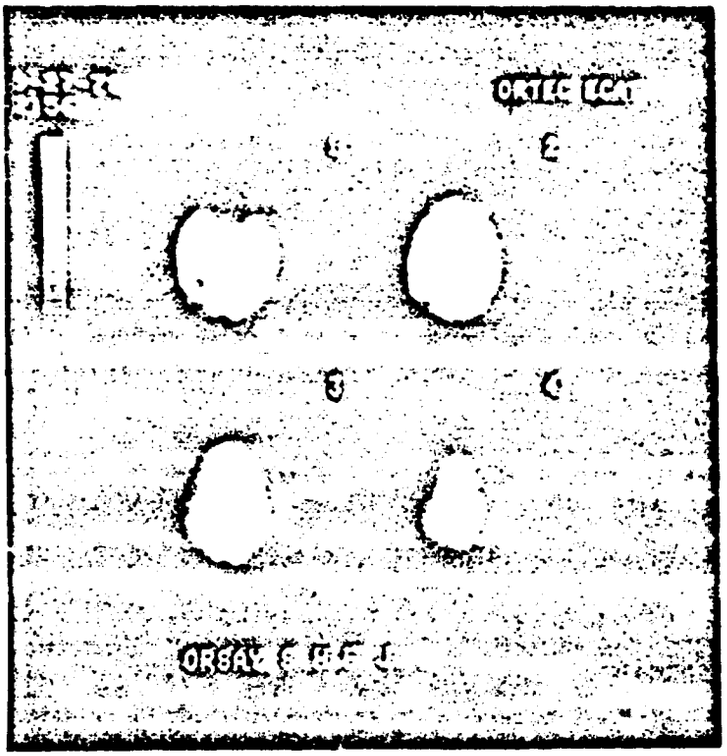


^{11}C Nicotine Brain Kinetics

XII



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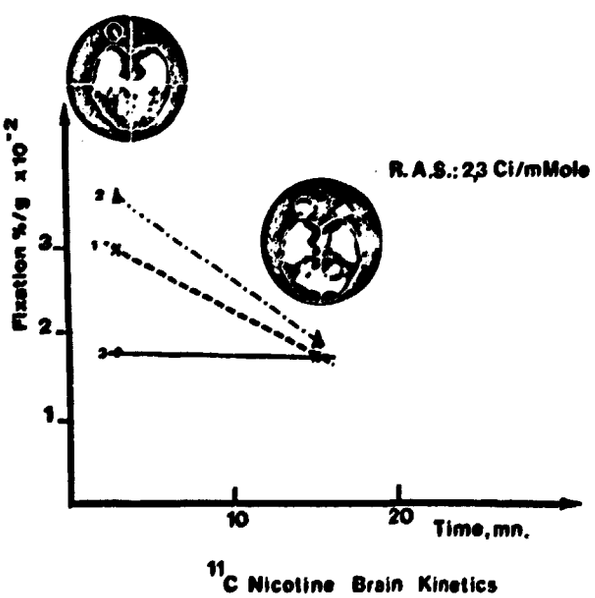

^{11}C Nicotine Brain Kinetics

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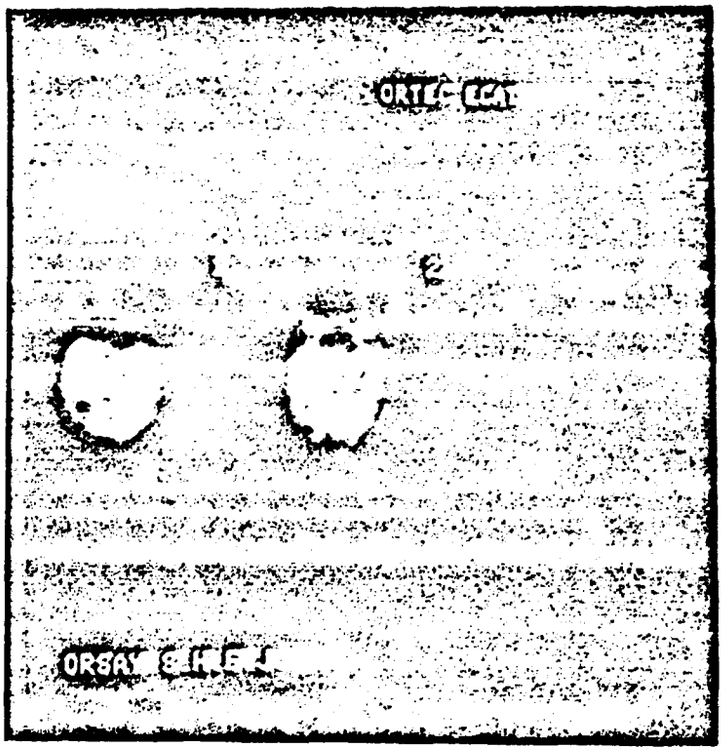

XV