RIJKSUNIVERSITEIT TE GRONINGEN



# CYCLOTRON PRODUCED SHORT-LIVED ISOTOPES IN NUCLEAR MEDICINE

## CARBON-11 AMINO ACIDS

PROEFSCHRIFT

TER VERKRIJGING VAN HET DOCTORAAT IN DE
WISKUNDE EN NATUURWETENSCHAPPEN
AAN DE RIJKSUNIVERSITEIT TE GRONINGEN
OP GEZAG VAN DE RECTOR MAGNIFICUS
DR. A. WATTEL IN HET OPENBAAR TE VERDEDIGEN OP
MAANDAG 8 JULI 1974
DES NAMIDDAGS TE 4 UUR

DOOR

**WILLEM VAALBURG**
geboren te Castricum

Druk: V.R.B.-Offsetdrukkerij – Kleine der A 4
Groningen – 1974.

PROMOTORES: PROF. DR. M.G. WOLDRING

PROF. DR. H. WYNBERG

STELLINGEN

1. Volgens Lathrop en medewerkers worden bij bestraling van water met protonen, met stikstof-13 gemerkte nitraten en nitrieten gevormd. De door hen gebruikte methode om deze radioaktieve produkten te reduceren tot $^{13}$N-ammonia is niet optimaal.

   **K.A. Lathrop, P.V. Harper, B.H. Rich, R. Dinwoodie, H. Krizek, N. Lembares, I. Gloria, Proc. IAEA-WHO Symp. Copenhagen, "Radiopharmaceuticals and labelled compounds" Deel I, blz. 471, Wenen (1972).**

2. Het is mogelijk met behulp van rectaal toegediend $^{13}$N-ammoniumacetaat de dominante richting van de portale bloedstroom vast te stellen.

3. Tricyclo $\left[5.3.0.0^{4,8}\right]$ deca-2,5,9-trieen staat ondanks een poging tot verbetering, nog steeds onder een onjuiste naam in de literatuur vermeld.

   **L.A. Paquette, M.J. Kukla, J. Amer. Chem. Soc. 94, 6874 (1972)**
   **J. Amer. Chem. Soc. 95, 988 (1973)**

4. De ontwikkeling van cyclotrons, uitsluitend voor de produktie van koolstof-11, moet gestimuleerd worden.

5. Nukleaire Geneeskunde dient te worden uitgeoefend door een multi-disciplinaire groep gekwalificeerde deskundigen.

6. Hulppredikers, evangelisten en catacheten aan wie men preekconsent verleend heeft, dient men in het licht van de reformatorische theologie zeker toestemming te verlenen om de sakramenten te bedienen, huwelijken in te zegenen en openbare geloofsbelijdenis af te nemen.

7. Predikanten moeten er rekening mee houden, dat een toenemend aantal beroepende gemeenten geen pastorie meer beschikbaar stelt.

8. Het streven, zowel nationaal als internationaal, naar uniformering van opleidingsduur en niveau van voortgezet onderwijs, mag niet ten gevolge hebben dat binnen het Hoger Beroepsonderwijs de tweejarige opleiding tot analist(e) als zelfstandige opleiding verdwijnt.

9. Om te zorgen dat het gebit van de Nederlandse bevolking in een zo natuurlijk mogelijke staat behouden blijft, dient de structuur van de tandheelkundige zorg verbeterd te worden.

10. In het kader van de wet op de Geneesmiddelenvoorziening is het eenvoudig te voorkomen dat aan kinderen beneden de leeftijd van 16 jaar suikerhoudende versnaperingen worden verstrekt of verkocht.

11. In een demokratie is de weg tussen het kastje en de muur geplaveid met rapporten en pamfletten.

**Stellingen behorende bij:**

**"Cyclotron produced short-lived isotopes in Nuclear Medicine"**
**"Carbon-11 amino acids"**

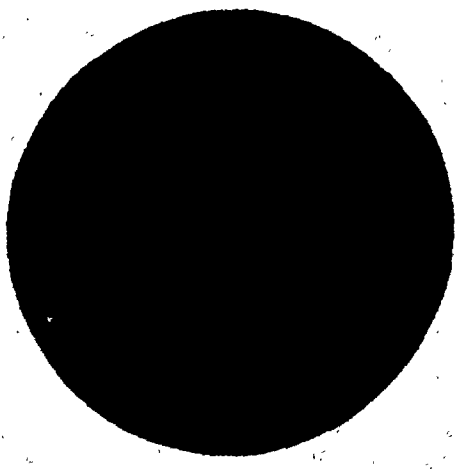
**W. Vaalburg** **Groningen, 8 juli 1974.**

Aan mijn ouders, door wie ik ben

Aan Jantie

Mariska

Henriëtte, voor wie ik ben



Dit proefschrift werd bewerkt in het Centraal Isotopenlaboratorium van het Academisch Ziekenhuis Groningen (Prof. Dr. M.G. Woldring), het Organisch Chemisch Laboratorium (Prof. Dr. H. Wynberg) en in het Kernfysisch Versneller Instituut (Prof. Dr. R.H. Siemssen) te Groningen.

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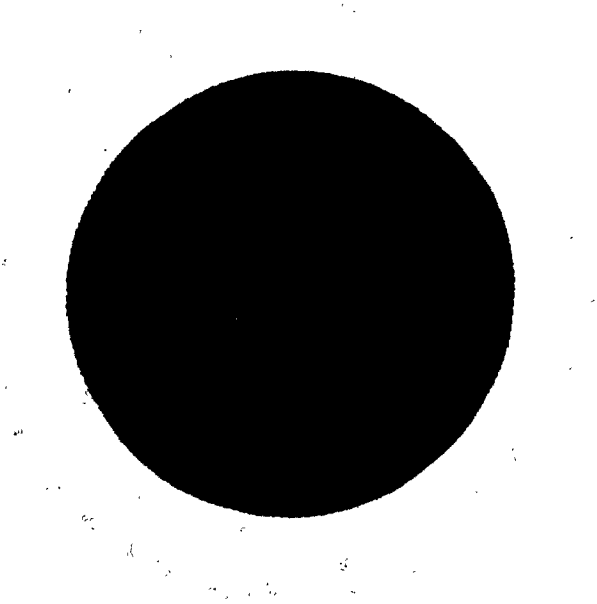


Chapter I.

# RADIOPHARMACEUTICALS LABELLED WITH SHORT-LIVED ISOTOPES

## *I.1. INTRODUCTION.*

In 1934 Curie and Joliots bombarded an aluminium foil with natural α-particles (1). After the α-source was removed, the irradiated foil emitted some kind of radiation, which decreased exponentially with time. This was the first time a radionuclide had been prepared. Shortly after this discovery several research groups produced new radionuclides. In 1940 Seaborg (2) could already publish a long list of known artificial radio-isotopes. By that time it was also recognised that the new radionuclides provided powerful tools for metabolic research. However the only production facility available for the pioneers was the cyclotron built by Lawrence in 1932 (3). Production of radionuclides with the early cyclotrons was only possible at great cost and effort.

In 1942 the first self-sustaining nuclear reactor became operative and a second, cheaper isotope production source became available.

In 1946 the Atomic Energy Commission of the USA announced that reactor-produced radioactive nuclides could be obtained for medical, industrial and scientific

research. From that time on the use of radionuclides for medical application increased gradually.

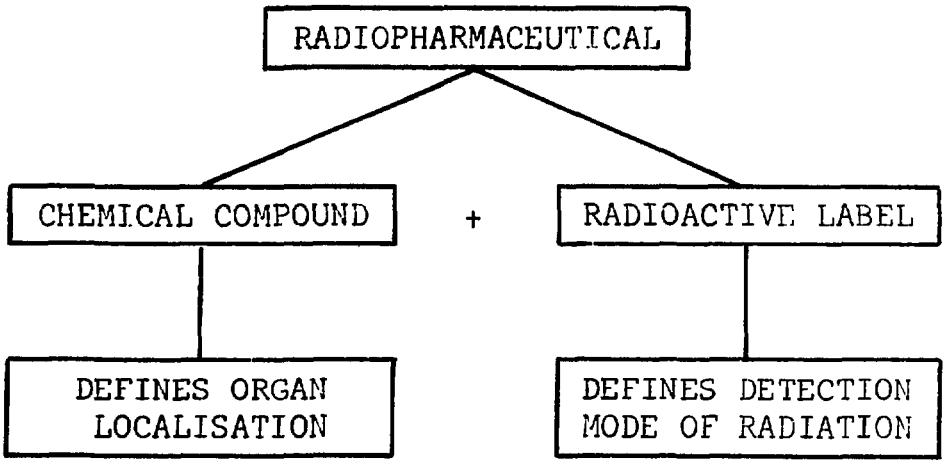
Today the specialisation which is engaged with radioisotopes in medicine is called Nuclear Medicine, a multidisciplinary branche of science. Nowadays radioisotopes are used in Nuclear Medicine for therapy and for diagnosis (4, 5). In therapy the destructive effect of radiation of the isotopes administered to the patient, is used. For diagnosis however, the information must be obtained as accurately as is necessary and this destructive effect minimised.

## *I.2. RADIOPHARMACEUTICALS.*

When a compound is administered to a human, the compound is used *"in vivo"*. When a material is not administered to a patient, but applied as an aid in an analytical technique, to determine for instance the concentration of a metabolic product in blood or urine samples, the material is used *"in vitro"*.

If, for medical application, a radioactive compound is used *in vivo*, it is called a radiopharmaceutical.

A radiopharmaceutical is defined by its chemical and by its radioactive properties. The chemical structure determines the mode of localisation of the material in the patient. A good radiopharmaceutical must have a good specificity for the organ under study. This means that the ratio between the accumulation in the organ and the accumulation in the surrounding tissue must be high. The




compound must have a good target/non-target ratio.

The type of radiation emitted by the radiopharmaceutical is dependent upon the nuclear properties of the radioactive label. For *in vivo* measurements it is necessary that the radiation can be detected outside the body of the patient (external measurements). Radiation which is absorbed within the body of the patient gives only radiation exposure and no diagnostic information. An "ideal" radioactive label for a radiopharmaceutical may not emit "useless" (not externally detectable) radiation.

Many diagnostic *in vivo* methods used in Nuclear Medicine are based on scintigraphic techniques. These are techniques by which an image of the tissue distribution of the radioactivity administered to the patient is obtained.

A different approach for diagnosis is to measure quantitatively the function of an organ by external measurements of the metabolic fate of a radiopharmaceutical (dynamic studies).

At the moment the resolution of scintigraphic procedures and the accuracy of dynamic studies are relatively low, because of the statistical limitation associated with the use of small amounts of radioactivity. The amount of radioactivity to be administered to the patient is limited by the maximum acceptable risk to the patient. This risk is determined by the absorbed radiation within the body: the absorbed dose (D). The absorbed dose can be expressed in the formula (6):

$$D = \left[51.2\ \bar{E}_{\beta} + 0.024\ \Gamma\ \bar{g}\right]\ {}_{t_1}\int^{t_2} C\ dt$$

where $\bar{E}_{\beta}$ is the average energy (in MeV) per desintegration of all locally absorbed radiation.

$\Gamma$ is the specific gamma ray constant in Röntgen per hour per millicurie at 1 cm.

$\bar{g}$ is the average geometrical factor for the absorber.

${}_{t_1}\int^{t_2} C\ dt$ is the cumulative concentration of the isotope in the absorber (μCi - hour per gram).

How can the radiation dose to the patient be minimised? $\bar{E}_{\beta}$ should be zero. This means, as already mentioned, that the applied radionuclide may not emit radiation which is not externally detectable. So desintegration of the nuclide may not be associated with the emission of $\beta^-$ particles, conversion electrons, Auger electrons or low energy photons. The radionuclide should be a

pure gamma-ray or positron emitter.

The quantity of radioactivity remaining in the body after the diagnostic information is obtained also delivers an unnecessary contribution to the absorbed radiation dose: the radioactivity must disappear as soon as possible. The disappearance occurs by biological clearance and by radioactive decay of the nuclide. The effective half-life ($t_{\frac{1}{2}\ eff.}$) of the radionuclide in the body depends on the biological half-life ($t_{\frac{1}{2}\ biol.}$) and the physical half-life ($t_{\frac{1}{2}\ phys.}$).

$$\frac{1}{t_{\frac{1}{2}\ eff.}} = \frac{1}{t_{\frac{1}{2}\ biol.}} + \frac{1}{t_{\frac{1}{2}\ phys.}}$$

Reduction of the effective half-life can be achieved by selecting the proper chemical form of the radiopharmaceutical and by labelling the compound with a short-lived isotope ($t_{\frac{1}{2}} < 15$ h).

## *I.3. PRESENT USE OF SHORT-LIVED RADIONUCLIDES IN NUCLEAR MEDICINE.*

As already discussed in I.2. radionuclides of short half-life ($t_{\frac{1}{2}} < 15$ h) are preferable as label for radiopharmaceuticals. A convenient way to make these isotopes available at long distances from the production site is the nuclide generator ("radioactive cow"). The principle of any nuclide generator (7) is a parent-daughter relation, whereby the short-lived daughter nuclide is, at time intervals, separated from its longer-lived

parent nuclide. However, the number of possible generator systems is limited. Bruce (8) lists parent-daughter relationships already used in the past for special purposes. Only a few are now used in general Nuclear Medicine practice (9).

## *I.4. THE USE OF CYCLOTRON-PRODUCED SHORT-LIVED RADIONUCLIDES.*

A different approach in making radioisotopes of short half-life available for medical application is to bring the source of production to the hospital.

Radionuclides can be produced by neutron irradiation with a nuclear reactor or by charged particle irradiation with a cyclotron (10). In general, neutron-excess nuclides are produced in a reactor and neutron-deficient nuclides with a charged particle accelerator. Neutron-excess radionuclides decay by emitting $\beta^-$ particles (not externally detectable) and neutron-deficient nuclides by electron capture or positron emission. Thus the production of short-lived radionuclides for medical purposes with a cyclotron must be prefered to the production mode with a nuclear reactor.

Nuclear reactions induced with charged particles very often result in product nuclides of a different element than the target nuclides. This implies that carrier-free products can be made with a cyclotron. With neutron capture (n, $\gamma$) reactions this is impossible. Carrier-free radionuclides are to be prefered in Nuclear Medicine

because radiopharmaceuticals labelled with these nuclides may be added to biological systems without significantly changing the physiology of the stable element or compound already present.

Another fact in favour of a cyclotron over a nuclear reactor as local production source of radionuclides of short half-life is that some of the short-lived isotopes ($^{11}C$, $^{13}N$, $^{15}O$), particularly useful for biomedical research can only be produced by bombardment with charged particles (11).

In conclusion may be said that the advantages of the use of cyclotron-produced short-lived isotopes for medical application above the use of longer-lived reactor-produced nuclides are:

a. the radiation dose to the patient is lower,
b. the type of emitted radiation of the isotopes is more useful for biomedical research,
c. more radioactivity can be administered to the patient within the safety limits, which results in more precise information,
d. the medical examination can be repeated within a short time,
e. some very useful isotopes can only be produced with a cyclotron
f. carrier-free products can be made,
g. the problems with waste disposal are minimal.

Advantages of short-lived isotopes obtained via a nuclide generator in respect to cyclotron products are:

a. these isotopes are easily available for every hospital
b. at every desired moment they can be eluted from the generator system.

The possibilities of accelerator-produced isotopes of short half-life were first fully recognised in England. In 1955 at Hammersmith Hospital in London, a cyclotron was installed exclusively for medical application (12, 13). From the beginning this group not only used their machine for isotope production but also for *in vivo* and *in vitro* neutron and charged particle activation analysis and for radiotherapy with fast neutrons (14).

As late as 1965 a second cyclotron for medical purposes was installed in the USA (15). At the moment the interest in the clinical application of this branch of Nuclear Medicine is growing rapidly (16). Cyclotrons are being installed in several hospitals around the world (17).

## *I.5. ORGANIC RADIOPHARMACEUTICALS LABELLED WITH CYCLOTRON-PRODUCED SHORT-LIVED ISOTOPES.*

Most of the radiopharmaceuticals in present use are labelled with radionuclides foreign to the body (an extreme example is the widespread application of $^{99m}Tc$, an isotope of an element that even does not naturally occur). This can be accounted to the fact that until

1969 the development of new radiopharmaceuticals was mainly based on the physical characteristics of the radionuclide used as label. Besides this the interest was focussed on preparing inorganic radiopharmaceuticals.

An expanding field of interest is the preparation of organic radiopharmaceuticals labelled with cyclotron-produced isotopes of short half-life (18, 19). Radionuclides which can be used for the labelling of these organic compounds are summarised in table I.5.

| Radionuclide | Half-life | Principal radiation (keV) |
|---|---|---|
| $^{11}C$ | 20.4 min | $\beta^+$ $\gamma$: 511 |
| $^{13}N$ | 10.0 min | $\beta^+$ $\gamma$: 511 |
| $^{15}O$ | 2.0 min | $\beta^+$ $\gamma$: 511 |
| $^{18}F$ | 110.0 min | $\beta^+$ $\gamma$: 511 |
| $^{34m}Cl$ | 33.0 min | $\beta^+$ $\gamma$: 511 : 148 |
| $^{74}Br$ | 42.0 min | $\beta^+$ $\gamma$: 511 |
| $^{78}Br$ | 6.5 min | $\beta^+$ $\gamma$: 511 : 614 |
| $^{121}I$ | 2.1 hr | $\beta^+$ $\gamma$: 511 : 212 |
| $^{123}I$ | 13.3 hr | $\gamma$: 159 |

*Table I.5.* Radionuclides for short-lived labelled organic radiopharmaceuticals.

A refinement in the range of radiopharmaceuticals will be the preparation of "true biological" labelled compounds: compounds which normally are involved in metabolism. One of the reasons that only a few are used nowadays is, that most of them do not contain elements other than carbon, hydrogen, oxygen and nitrogen. The chart of nuclides shows that of these elements only a limited number of externally detectable isotopes with useful half-lifes (1 min < $t_{\frac{1}{2}}$ < 15 hr) are known ($^{11}C$, $^{13}N$, $^{15}O$). They all emit positrons. Because of the subsequent positron-electron annihilation, two photons of 511 keV energy are emitted at 180° with respect to each other.

The simultaneity of the two photons permits the use of coincidence counting techniques in scintigraphy. These techniques improve detection sensitivity and facilitate the determination of the spatial origin of the radiation.

A disadvantage of the positron emitters is the high energy of the photons. The sensitivity of the scintigraphic instrumentation at this moment available (gamma camera) is relatively low. Besides this the radiation is difficult to collimate.

The occurrence of the element carbon in nearly every biological compound and the half-life of carbon-11 makes this radioisotope potential the most useful radionuclide in Nuclear Medicine.

The first clinical application of carbon-11 was

reported in 1960 by the Hammersmith group. They used $^{11}CO$ and $^{11}CO_2$ for lung function studies (20) and for labelling red blood cells (21). In 1967 Myers and Hunter (22) discussed the possibilities of $^{11}C$-labelled organic compounds for medical diagnosis. In 1969 Winstead (23) reported the synthesis of a $^{11}C$-compound (sodium $^{11}C$-benzoate) with the aim to test the material as radiopharmaceutical. Since then the interest in carbon-11 chemistry has grown rapidly (19).

It is remarkable that the synthesis and the biochemical use of the first $^{11}C$-labelled compound had already been reported in 1939 (24). After 1943 (25) hardly any work was done in this field until 1969. The reason was that during World War II the $\beta^-$-emitter carbon-$_{14}$, with a half-life more convenient for biochemical work, became available. The growth of Nuclear Medicine and the increased availability of cyclotrons renewed the interest in the positron emitting carbon-$_{11}$ isotope. In the future a further expansion may be expected.

## *I.6. AIM OF THIS WORK.*

Unlike many other internal organs, visualisation of the pancreas by whatever means has been very unsuccessful. This makes the diagnosis of diseases affecting this organ rather difficult. For this reason the development of a pancreatic scanning agent is an important research area in Nuclear Medicine.

Blau (26) postulated in 1961 that the administration of a precursor amino acid labelled with a suitable gamma-ray emitting isotope might result in a sufficiently high concentration of radioactivity in the pancreas to permit visualisation by scintigraphic techniques. His idea was based on the fact that the pancreas synthesises proteins at a very high rate due to the continuous production of digestive enzymes. Blau used a seleno-analogue of methionine because no other γ-ray emitting isotope for the synthesis of an useful amino acid was available. With access to a cyclotron the range of gamma-ray emitting isotopes to label amino acids is more extended. The isotopes of interest for labelling these compounds are $^{11}C$, $^{13}N$, $^{18}F$.

Our aim is to prepare DL-α-phenylglycine-1-$^{11}C$ and DL-α-phenylalanine-1-$^{11}C$ and to test these compounds as pancreas scanning agents.

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Chapter II.

# THE PRODUCTION OF CARBON-11

## *II.1. INTRODUCTION.*

### *Nuclear reactions.*

The entrance of any particle into a nucleus results in a rearrangement of nuclear material to form a new substance. This process is called a nuclear reaction. Bombarding with a sufficient number of projectiles will result in the accumulation of a quantity of the product nuclide. A nuclear reaction evidently proceeds in two stages. The first is the formation of a very unstable "compound nucleus" containing all the material of both target and bombarding nuclei and the second a very prompt rearrangement to a more stable state with the emission of energy and, frequently, of particles. A compact notation has been devised, which can be illustrated by the following example. When copper-65 is bombarded with deuterons nickel-63 is formed; an alpha particle is expelled.

$$^{65}Cu\ (d,\ \alpha)\ ^{63}Ni$$

The target nucleus is placed before the parentheses, the bombarding particle just inside, the expelled particle or radiation next, and finally outside the parentheses the product nucleus.

*Recoil reactions.*

The energy released in the reaction is carried away by the emitted particle or radiation and by the newly formed atom. The product nucleus is called a "hot atom" because it is in an unusually high energy state (this energy is called recoil energy). The recoil energies can vary from 0.1 eV or less to several MeV. The energy of the formed atom is in most cases sufficiently high to break the bond with the mother molecule. The "hot atom" moves into the surrounding material. The increased energy of the atom is often manifest in the form of electronic excitation, ionisation and a high kinetic energy. Through electronic interactions and scattering phenomena the atom is slowed down and reaches an energy state, where elastic and inelastic collisions can occur. Before the atom is in thermal equilibrium with its surrounding, it reacts chemically with the surrounding material to give one or more stable products. This process is called a "hot-atom reaction" or a "recoil reaction". Because the energy of the atom, at which a hot-atom reaction takes place, is much higher than the energy of a thermal atom, it undergoes a reaction that will be different from the reaction of a thermal atom. Some criteria are applied to set a recoil reaction apart from a thermal reaction. A recoil reaction is:

- temperature insensitive
- phase independent
- dependent on radical scavengers
- dependent on moderators (e.g. inert gases).

General information about hot-atom chemistry can be found in a review article by Wolf (1).

*Radiolysis.*

Not only a recoil process takes place during bombardment. To induce a nuclear reaction a large amount of radiation is required. It is unavoidable that the target material is damaged by this radiation or by the recoiling atom (radiolytic decomposition). Impurities in the bombarded material play in this case an important role during irradiation. Impurities, for example, can be excited and can react with the target material or with the recoiling atom. Certainly they affect the spectrum of compounds obtained after irradiation, particularly when the target compound is bombarded in the gas phase.

*End products.*

When simple, high specific activity or carrier-free compounds, as for instance $^{11}CO$, $^{11}CO_2$, $H^{11}C{\equiv}CH$, $^{123}ICl$ or $NaB^{18}F_4$ are required, use can be made of recoil and radiolytic processes. By selecting the proper target material, nuclear reaction and irradiation conditions the chemical form of the radioactive products can to some extent be controlled.

Preparation of a labelled complex organic compound, for application in Nuclear Medicine, by bombardment of a complex organic target material however, is not very practical because a highly impure product with a low specific activity will be obtained. For that reason the

preparation of a short-lived labelled organic radiopharmaceutical is often a two step process:

a. recoil or radiolytic preparation of a radioactive precursor.
b. chemical or biochemical synthesis.

## *II.2. PRODUCTION OF A RADIOACTIVE PRECURSOR.*

### *Production.*

The optimal production method of a radioactive precursor depends on the following parameters:

a. cyclotron parameters
   - accelerated particles
   - available energy
   - available beam current
b. reaction cross-section
c. half-life of the produced radionuclide

The yield A of the isotopes produced depends on the type of the nuclear reaction (the excitation function), the beam current I, the target thickness, the chemical form of the target material, the bombarding time t, and the decay constant $\lambda$ of the nuclide. All these variables are expressed in the formula:

$$A = R\ I\ (1 - e^{-\lambda t})$$

where, besides the symbols already introduced, R is the production rate.

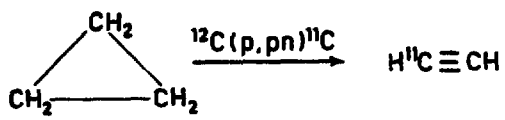
### *Radionuclidic purity.*

The radionuclidic purity of the product is determined by the relative yield of the nuclear side reactions under

the bombarding conditions, the purity and the thickness of the target material and the differences between the half-lives of the product and the undesired radionuclides. In most cases purification of the bombarded material by a chemical or physical method is necessary. When the impurities are isotopic with the product an optimum in the production conditions must be found to minimise the yield of side reactions.

*Specific activity.*

As discussed in I.4. the preparation of carrier-free products is often desirable, so target material and product may not in general be isotopic. Carrier-free products are principally produced by nuclear transmutation reactions. A non-transmutation reaction is only useful if the chemical forms of the target compound and the product are different. In that case separation is possible, for instance with a chromatographic technique. The chemical difference of target and product may be the result of a hot-atom process. An example of the production of a carrier-free compound by a non-transmutation reaction is the preparation of acetylene-$^{11}C$ using the (p, pn) reaction on cyclopropane (2).



## *II.3. TARGET MATERIAL.*

A single isotope in the elemental form is, from the stand-point of yield of a radioactive product per unit weight of bombarded material, the most desirable. However, most elements are poly-isotopic and the use of enriched material is not always possible. Considerations other than those based on yield and purity of the product also play a role in the choice of the chemical and physical composition of the target material. One of the most important factors limiting the choice is the problem of dissipating the heat input during the bombardment. The material must be able to withstand the bombarding beam without significant or undesired decomposition. An other point which limits the available target material is that, with a proper choice of the material to be bombarded, the chemical form of the induced radioactive products can be controlled (see II.1.).

For every production method the isotopic purity, the chemical purity and also the physical form of the target material must be specified in relation to particle energy and target thickness. For the production of short-lived isotopes it is also important that the product can rapidly be separated from the bombarded material to limit loss of radioactivity by decay.

## *II.4. TARGET SYSTEMS.*

To irradiate a compound in a solid, liquid or gaseous form with charged particles a container must be constructed for the sample to be bombarded. Such a container is called a target system. For efficient isotope production it is very important that a suitable system be designed. Vonberg (3) gives the main factors determining the construction of a target system:

- the physical and chemical properties of the target material,
- the cooling provision to control the temperature rise of the material under bombardment,
- the target thickness that will maximise the yield of the required isotope and minimise the yield of possible impurities,
- the recovery method of the required isotope from the target material.

The time factor is important in work with short-lived isotopes and it is necessary to reduce the post-irradiation time. The recovery of the product must be rapid and quantitative. The product must be radiochemically pure. A target system from which the produced radioactive material can be recovered in the gasphase (gas flow target system) fulfils these conditions best:

- the chemical treatment of the product can proceed simultaneously with the bombardment.
- the radiochemical impurities are reduced because

non-volatile radioactive material remains in the target system.

- radiation damage of the product is reduced because the product is continuously removed.
- the circulating gas flow can be used for cooling the target system.

## *II.5. THE PRODUCTION OF CARBON-11.*

*Nuclear reactions.*

The first production of carbon-11 was reported in 1934. Crane (4) bombarded boron with protons and induced carbon-11 by the reaction

$$^{10}B\ (p,\ \gamma)\ ^{11}C.$$

Since that time a number of nuclear reactions with $^{11}C$ as product nuclide is described. In most cases charged particles are used as bombarders. A nuclear reaction with neutrons is:

$$^{12}C\ (n,\ 2n)\ ^{11}C.$$

However, the 18.7 MeV threshold of this reaction is too high for making carbon-11 with a nuclear reactor. With an electron linear accelerator (Linac) carbon-11 can be produced through the

$$^{12}C\ (\gamma,\ n)\ ^{11}C$$

reaction, using the "Bremsstrahlung" produced by the accelerated electrons (5).

When a cyclotron, which can accelerate protons, deuterons, helium-3 and α-particles is available, the best production methods are based on the bombardment of

carbon, nitrogen or boron. In table II.5. the most important nuclear reactions are summarised. In figure II.5. the excitation curves of these reactions and of competing reactions producing nitrogen-13, are given. The data are obtained from Landbolt and Börnstein (6). When we look at the cross-sections the best yields are obtained with the reactions:

A. $^{10}B$ ($^{3}He$, pn) $^{11}C$.

B. $^{12}C$ ($^{3}He$, $\alpha$) $^{11}C$.

C. $^{14}N$ (p, $\alpha$) $^{11}C$.

*Reaction A.*

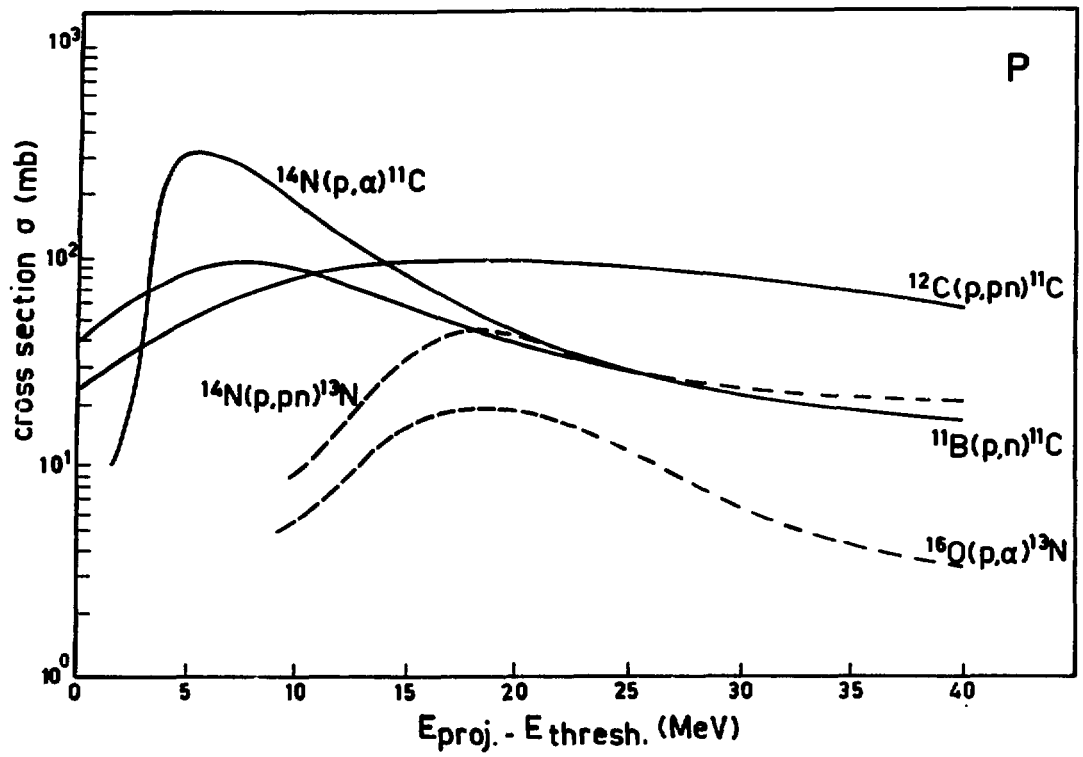
Boron-10 cannot be irradiated as a gas. This means that quantitative recovery of the produced $^{11}C$, from the target material is difficult (10).

*Reaction B.*

The reactions with carbon-12 as target nucleus suffers from the disadvantage that the target material and the product are isotopic. Carrier-free products cannot be made by these reactions, unless chemical separation is possible (see II.2.).

*Reaction C.*

The production of carbon-11 by bombardment of nitrogen-14 with protons is the best method. The nuclear reaction has a high yield and moreover, use can be made of the advantages of a gas flow target system (see II.4.). We used this reaction for the production of $^{11}CO_2$.



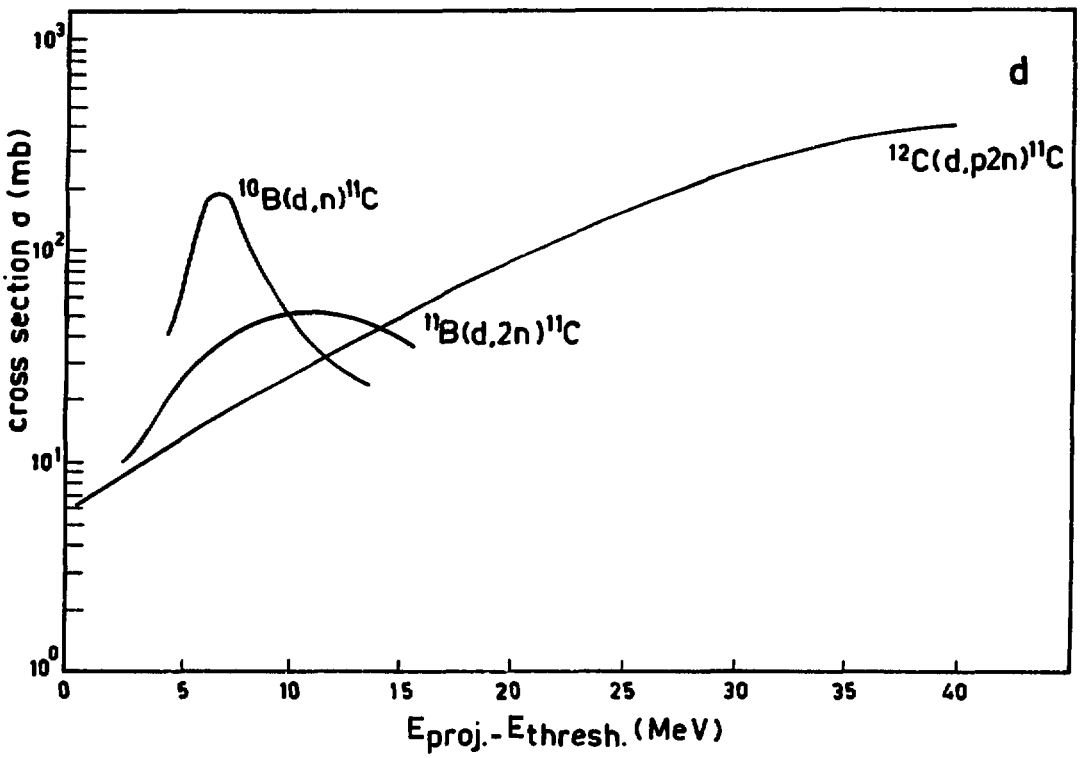




*Figure II.5.a.* Excitation functions of proton and deuteron reactions for the production of carbon-11 and the excitation functions of the competing reactions producing nitrogen-13.

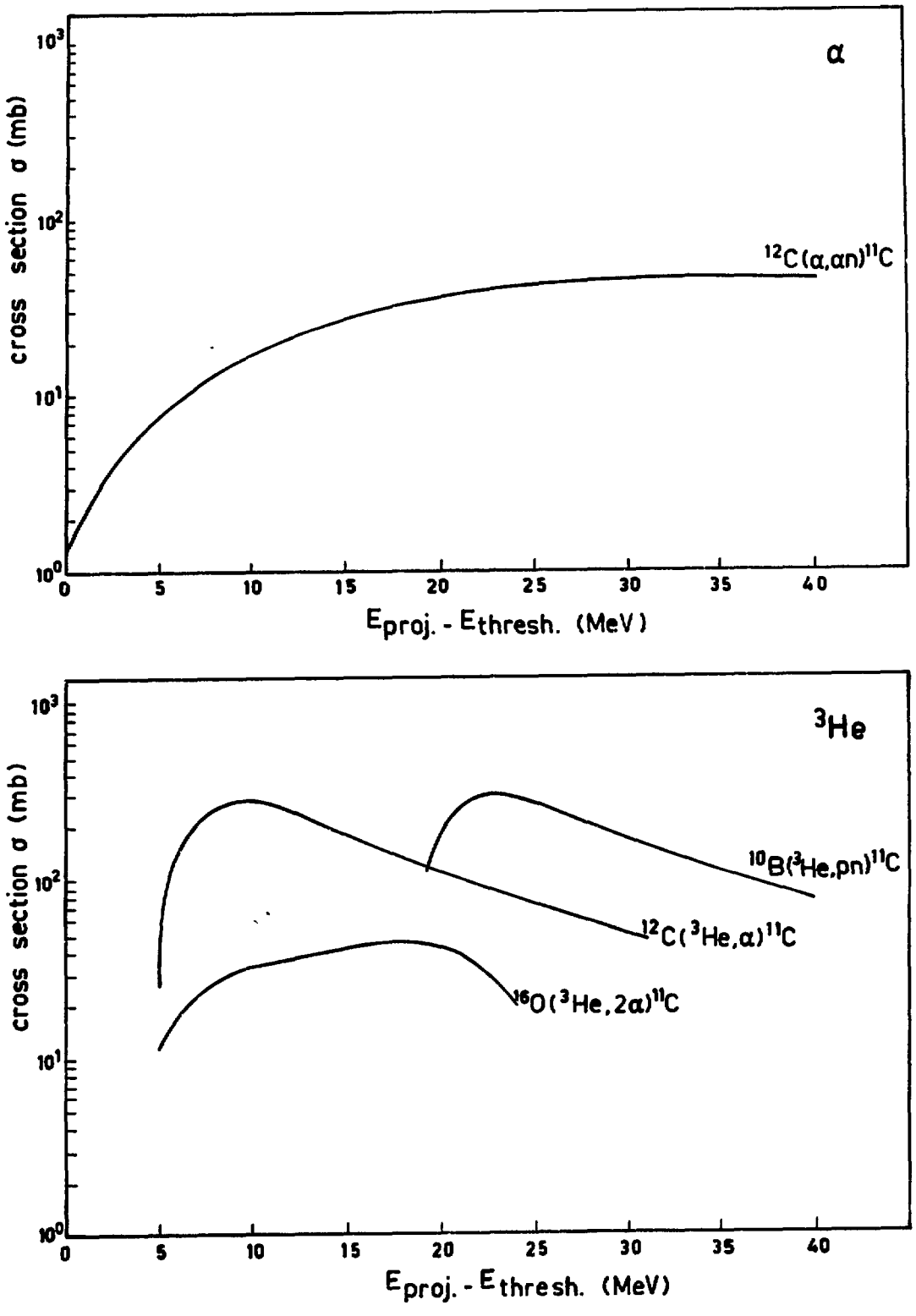



*Figure II.5.b.* Excitation functions of the α- and $^{3}He$-reactions for the production of carbon-11.

| beam | Reactions | Q-value (MeV) | $E_{thresh.}$ Threshold energy (MeV) | $\sigma_{max}$ Maximum cross section (mb) |
|---|---|---|---|---|
| p | $^{11}B$ (p, n) $^{11}C$ | -2.8 | 3.0 | 100 |
| | $^{12}C$ (p, pn) $^{11}C$ | -18.7 | 20.3 | 95 |
| | $^{14}N$ (p, $\alpha$) $^{11}C$ | -2.9 | 3.1 | 250 |
| d | $^{10}B$ (d, n) $^{11}C$ | +6.5 | 0 | 180 |
| | $^{11}B$ (d, 2n) $^{11}C$ | -5.0 | 5.9 | 48 |
| | $^{12}C$ (d, p2n) $^{11}C$ | -20.9 | 24.4 | |
| $^{3}He$ | $^{10}B$ ($^{3}He$, pn) $^{11}C$ | +1.0 | 0 | 285 |
| | $^{12}C$ ($^{3}He$, $\alpha$) $^{11}C$ | +1.9 | 0 | 260 |
| $^{4}He$ | $^{12}C$ ($\alpha$, $\alpha$n) $^{11}C$ | -18.7 | 25.0 | 48 |

*Table II.5.* **The most important charged particle reactions for the production of carbon-11.**

## *II.6. KNOWN $^{11}C$-LABELLED PRECURSORS FOR CHEMICAL SYNTHESES.*

Only a few methods for the direct cyclotron production of carbon-11 labelled synthetic precursors are reported in the literature. The earliest method is the production of carrier-free $^{11}CO$ and $^{11}CO_2$ by the deuteron bombardment of boron oxide. This method is described by Ruben (7), Buckingham (8), Welch (9) and Clark (10). When the boron oxide is irradiated, $^{11}CO$ is probably formed by a recoil reaction and a part of the recoil product is radiolytically oxidized to $^{11}CO_2$. When the proper beam conditions are selected, the $B_2O_3$ just melts and a mixture of $^{11}CO$ and $^{11}CO_2$ can diffuse out of the target material. With a circulating gas the radioactive products are swept out of the target system and the mixture reduced (for $^{11}CO$-production) respectively oxidized (for $^{11}CO_2$-production) by an "on line" procedure. In principle the same target system is employed (11) for the production of $^{11}CO_2$ via:

$$^{11}B\ (p,\ n)\ ^{11}C.$$

Wolf (11) and Finn (12) reported the production of $^{11}CO_2$ by proton bombardment of a nitrogen gas flow (which contains a trace amount of oxygen). The advantages of a gas flow target system and the high yield make this method very attractive.

Carbon-11 labelled potassium cyanide can be prepared from $^{11}CO_2$ by the reaction (13):

$$^{11}CO_2 + K + NH_3 \longrightarrow K^{11}CN$$

$Na^{11}CN$ is produced by direct irradiation of NaCN with protons (14) using simultaneously the reactions:

$$^{14}N\ (p, \alpha)\ ^{11}C \quad \text{and} \quad ^{12}C\ (p, pn)\ ^{11}C.$$

The product however is not carrier-free.

The production of $H^{11}CN$ by bombardment of a mixture of $N_2$ and $H_2$ in a gas flow target system is investigated by Finn (14) and Lamb (15). While the recovery of the produced $H^{11}CN$ from the target system is troublesome Christman (16) modified the method. When the radiation dose to the system is more than 0.5 eV/molecule, the produced $H^{11}C{\equiv}N$ is converted in the target system to $^{11}CH_4$. The radioactive methane can quantitatively be recovered from the target system. When the bombarded gas is passed over a catalyst at elevated temperature, the $^{11}CH_4$ is converted back to $H^{11}CN$. They claim a reliable method with a high yield of carrier-free product.

The preparation of $H^{11}C{\equiv}CH$ from $^{11}CO_2$ was described by Cramer (17) in 1941. Myers (18) prepared acetylene-$^{11}C$ (not carrier-free) by the irradiation of $CaC_2$ with $^3He$. In spite of a high cross-section (see table II.5.), he reports a low yield. The direct cyclotron production of carrier-free acetylene was described by Finn (2). He bombarded cyclopropane with protons and separated the radioactive product "on line" from the irradiated cyclopropane gas by a molecular sieve.

## *II.7. DESIGN OF A TARGET SYSTEM FOR THE PRODUCTION OF $^{11}CO_2$.*

$^{11}CO_2$ was produced by bombarding nitrogen gas (mixed with oxygen) with a proton beam. A continuous gas flow target system was used (see figure II.7.). The system consists of a 100 cm aluminium pipe with a diameter of 5 cm. The stainless steel back-plate (glued to the aluminium pipe with Araldite ®) is water-cooled. The beam enters the target system via a 4.5 cm diameter window assembly. The window assembly is composed of an aluminium front-plate (which is glued to the aluminium pipe with Araldite ®), a cross-linked polystyrene insulator, a 0.5 mm thick aluminium window foil and an aluminium front-flange. The window foil is fixed with two O-rings between the front-flange and the polystyrene insulator.

A gas inlet and a manometer are located near the window assembly. The bombarded gas is removed from the target system via an outlet near the back-plate. The gas pressure in the system and the flow rate can be controlled by needle valves. Nylon tubes (Nylaflow ®, diameter 6 mm) are used to transport the bombarded gas to the radiochemistry laboratory.

At the end of the beam line the vacuum of the cyclotron is separated from the atmosphere by a 0.1 mm thick aluminium foil. The gas flow target system is connected to the end-flange of the beam pipe. The target system and the end-flange are held together by evacuating a

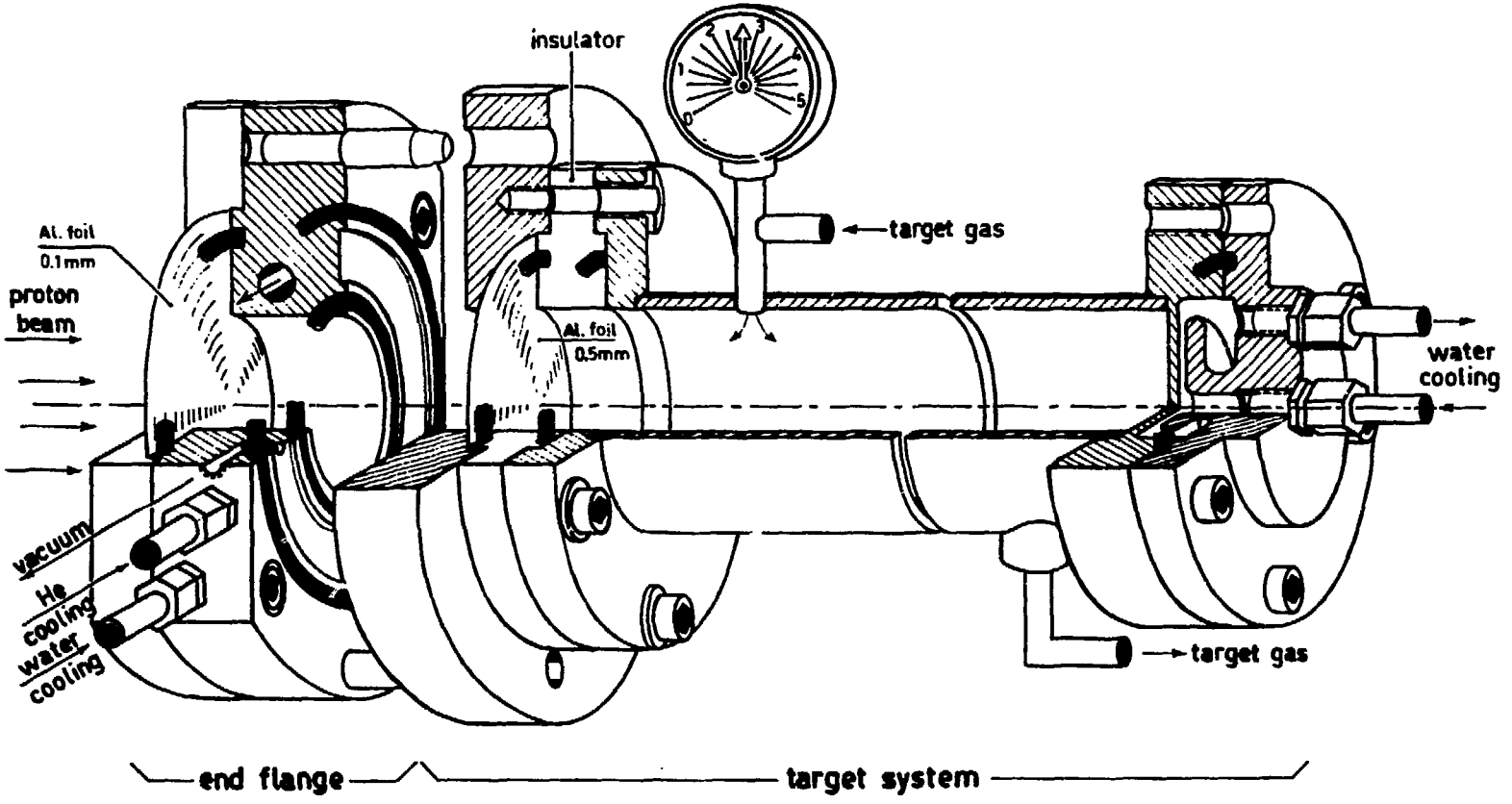



*Figure II.7.* Gas flow target system for the production of $^{11}CO_2$.

depression in the end-flange of the beam line. Because a long target system is used, support at the back end of the system is necessary. Between the foil of the target system and the foil of the beam line a flow of helium can be applied for cooling. The foil of the beam line can be cooled with water.

## *II.8. $^{11}CO_2$ PRODUCTION - EXPERIMENTAL AND RESULTS.*

The irradiations were carried out with the external proton beam of the 280 cm AVF cyclotron (Philips) of the Kernfysisch Versneller Instituut (State University, Groningen). The design specificiations of the cyclotron are given in table II.8.1.

| Beam | Energy (MeV) | External current (μA) |
|---|---|---|
| $p$ | 5 - 70 | 25 |
| d | 10 - 65 | 25 |
| $^3He$ | 15 - 165 | 15 |
| α | 20 - 130 | 15 |

***Table II.8.1.*** **Design specifications of the cyclotron at Groningen.**

The internal beam is extracted from the cyclotron by an extraction magnet and focussed by quadrupole magnets. A switching magnet guides the beam into the line which

is installed for isotope production. The shape of the beam can be monitored at two places by television viewing targets, which can be moved into and out of the beam by remote control. With one of the television viewing targets the beam spot can be displayed and the beam current measured, just before the beam enters the gas flow target system. We normally used a beam spot of 1 $cm^2$.

*The gas flow system.*

The gas flow system is shown in figure II.8.1. The target gas was introduced into the target system through nylon tubing via a needle valve. The effluent gas from

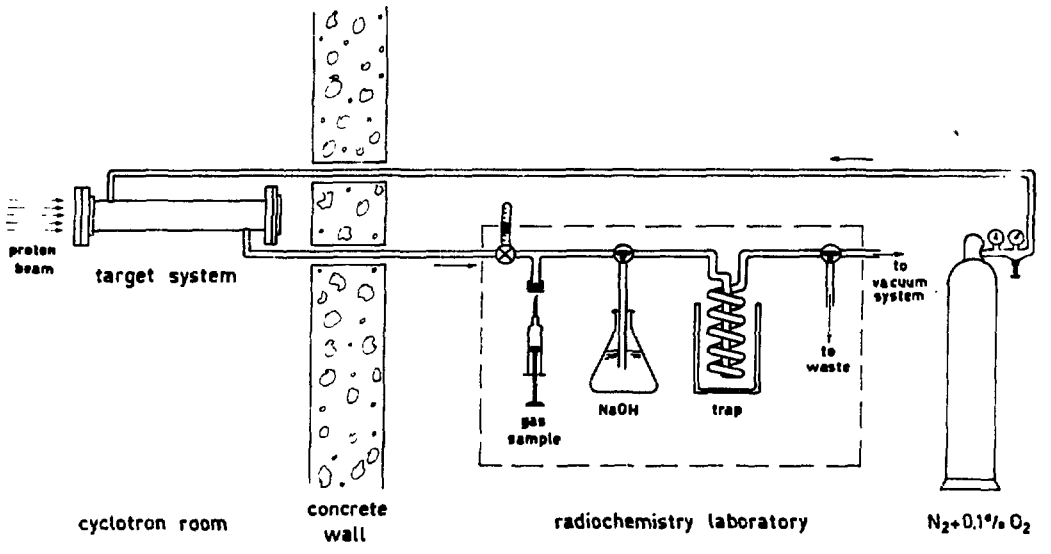



*Figure II.8.1.* **Gas flow system for the production of $^{11}CO_2$.**

the target system was transported to the radiochemistry laboratory also through nylon tubing via a needle valve and a flow ratemeter. The distance between the target

system and the laboratory is about 40 meter. The pressure of the gas under bombardment and the flow rate through the target system could be regulated by the needle valves.

Following the recommendations of the Hammersmith group, $^{133}Xe$ was used to determine the wash-out pattern of the produced radioactivity from the target system. A 1 ml $^{133}Xe$-gas sample was injected into the gas flow via a

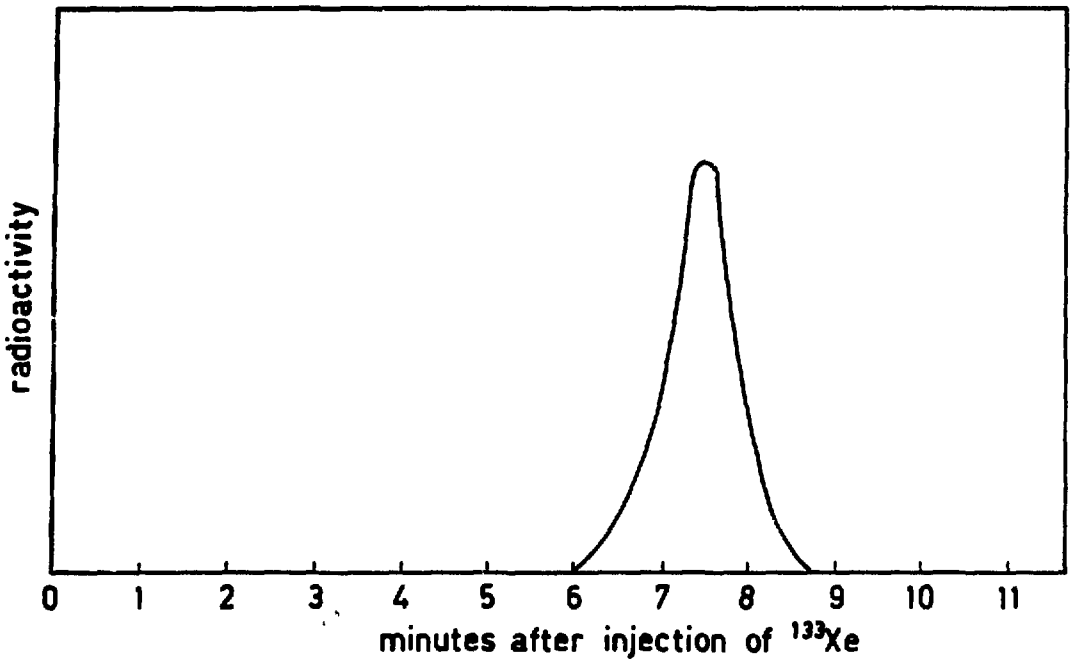



*Figure II.8.2.* **$^{133}Xe$-distribution of the effluent gas of the target system at a target gas pressure of 2 atm.**

septum installed in the flow system, just before the gas inlet of the target system. The $^{133}Xe$-radioactivity of the effluent gas was recorded in the radiochemistry laboratory by a scintillation detector-amplifier-ratemeter-recording system. In figure II.8.2. the $^{133}Xe$-distribution in the target gas flow is given. At a flow rate of

500 ml/min. and a gas pressure of 2 atm., 7.5 minutes elapsed between the injection of the $^{133}Xe$ and the moment the maximum amount of $^{133}Xe$ in the gas flow was recorded. At a gas pressure of 3 and 4 atm. the maximum amount was measured respectively after 8.2 minutes and 9.5 minutes. From these figures may be concluded that, with a target gas pressure of 3 atm. 25 % of the produced $^{11}CO_2$ is lost by decay during transportation.

For the $^{11}CO_2$ yield determinations it was possible to bubble the bombarded gas through a NaOH-solution. In the flow system a septum also was installed. Via this septum gas samples could be withdrawn for gas-chromatographic assay of the activated gas. When the $^{11}CO_2$ produced was used for organic syntheses, the gas was collected from the flow in a copper spiral by cooling with liquid nitrogen. The spiral was connected with a manifold of a vacuum system. When the spiral was evacuated at $-180^{\circ}$, followed by heating to about $80^{\circ}$, it was possible to distil the $^{11}CO_2$ nearly quantitatively into any reaction vessel connected with the manifold.

*Target gas.*

As target gas $N_2$ mixed with 0.1 % $O_2$ was used. The gas was obtained in a premix tank from Hoek-Loos (Amsterdam). The composition of the gas was:

$N_2 > 99.8$ %, $O_2 = 0.1$ %, $Ar < 0.1$ %,
$H_2O < 50$ ppm, other impurities < 10 ppm.

*Analysis.*

The analysis of the irradiated gas was carried out on a Packard 7400 gaschromatograph. The radioactivity of the effluent gas of the thermal conductivity detector was analysed with a lead-shielded NaI-scintillation detector, a ratemeter and a recorder. The chromatography columns used were:

a). 4 meter, $\frac{1}{4}$ inch stainless steel filled with 30-60 mesh activated charcoal (Chrompack, Holland). Elution sequence $N_2$, CO and $CO_2$.

b). 4 meter, $\frac{1}{4}$ inch stainless steel filled with 80-100 mesh Poropak® Q. Elution sequence $N_2$ + CO, $CO_2$.

The gaschromatograph was programmed, so that after injection of the gas sample the temperature of the column oven increased from $100^{\circ}$ to $210^{\circ}$ with a programmed rate of $30^{\circ}$ per minute and a final temperature holding period of 5 minutes. Helium was used as carrier gas.

While the concentration of $^{11}CO$ and $^{11}CO_2$ in the bombarded gas is too low to give a response of the thermal conductivity detector, compound identification was achieved by adding CO and $CO_2$ as carrier to a 1 ml sample of the bombarded gas, before injection on the columns. In figure II.8.3. typical radiogaschromatograms are given.

The $^{11}CO_2$ yield under different bombarding conditions was determined by bubbling the activated gas during 5 minutes through 10 ml of a 1 N NaOH solution. The absorbed radioactivity was measured in an ionisation chamber.

In table II.8.2. the radioactive composition of the

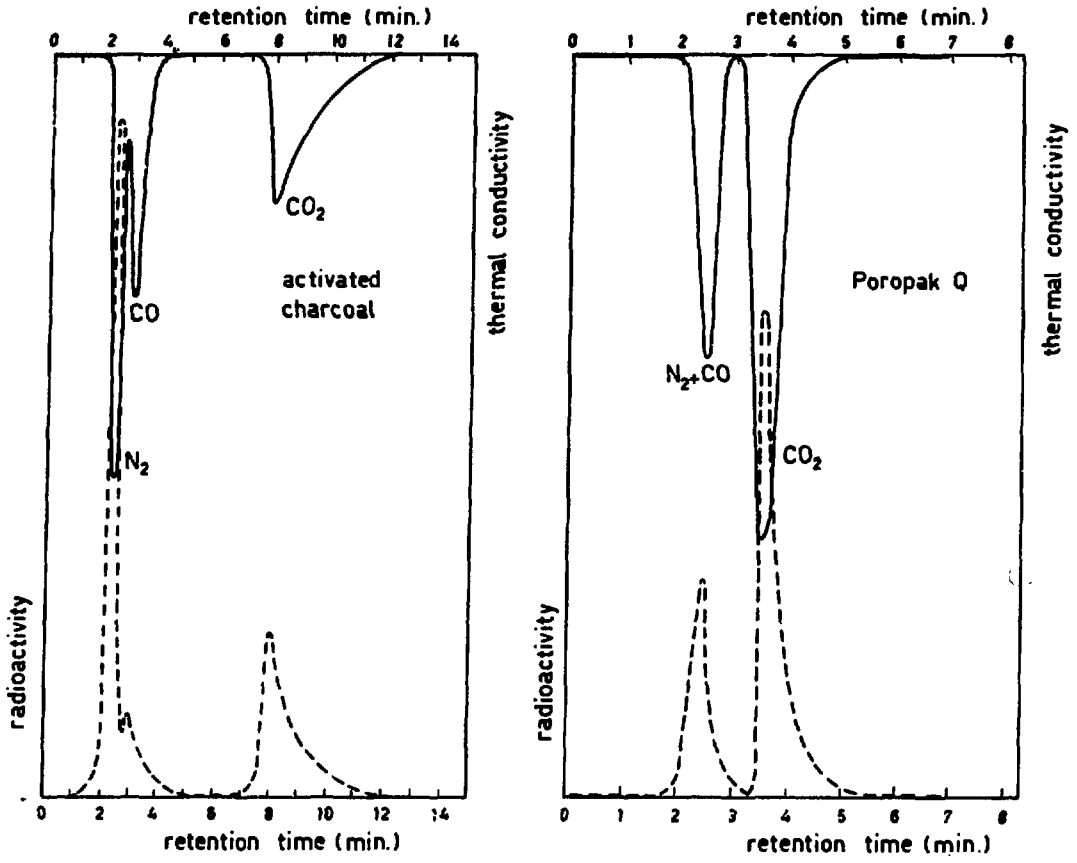



*Figure II.8.3.* Radiogaschromatograms of the bombarded target gas. Dashed line is the radioactivity distribution, solid line the thermal conductivity of the effluent gas.

bombarded gas and the $^{11}CO_2$ yield under different bombarding conditions are given. All the irradiations were carried out with a beam spot of 1 $cm^2$ and a target gas flow of 500 ml/min.

Under all the irradiation conditions used the amount of $^{11}CO$ was less than 1 % of the induced radioactivity in the target gas. When the incident proton energy was

| Target gas pressure (atm.) | Energy degradation in target gas (MeV) | Yield as % of total gas activity | | $^{11}CO_2$ yield (mCi/μA.min.) |
|---|---|---|---|---|
| | | $^{13}N_2$ + $^{11}CO$ | $^{11}CO_2$ | |
| 1.3 | 16.8 - 12.0 | 24 | 76 | 1.0 |
| 2.0 | 16.8 - 8.6 | 24 | 76 | 1.3 |
| 3.0 | 16.8 - 0 | 22 | 78 | 1.5 |
| 1.1 | 14.5 - 10.0 | 31 | 69 | 0.9 |
| 1.2 | 11.1 - 3.5 | < 1 | > 99 | 0.7 |
| 2.0 | 11.1 - 0 | < 1 | > 99 | 0.7 |
| 3.0 | 11.1 - 0 | < 1 | > 99 | 0.7 |

*Table II.8.2.* Composition of target gas and yield of $^{11}CO_2$ after bombardment.

kept below 11.3 MeV, the threshold energy of the $^{14}N$ (p, pn) $^{13}N$ reaction, less than 1 % of the total radioactivity in the target gas was in the form of $^{13}N_2$. This small amount of $^{13}N_2$ was induced by the reaction $^{16}O$ (p, $\alpha$) $^{13}N$ (threshold energy 5.5 MeV). Only a little $^{13}N_2$ was produced because the maximum cross section (at 17.5 MeV) of the last mentioned nuclear reaction is 19 mb. Moreover only 0.1 % $O_2$ was present in the target gas.

With 11.1 MeV incident proton energy the yield of $^{11}CO_2$ was 0.7 mCi/μA.min. Theoretically an increase in target gas pressure should result in higher yields. In our system however the $^{11}CO_2$ yield remained constant when the pressure increased.

With an incident energy of 16.8 MeV and a target gas pressure of 1.3 atm. we obtained a $^{11}CO_2$ yield of 1.0 mCi/μA.min. We could improve the yield to 1.5 mCi/μA.min. by increasing the target gas pressure up to 3.0 atm. Under these bombarding conditions 21 % of the total radioactivity was in the form of $^{13}N_2$ and less than 1 % in the form of $^{11}CO$. More experiments have to be done to optimise the production of $^{11}CO_2$, for instance by using a higher proton energy and target gas pressure. We have an indication that technical $N_2$ can be used as target gas. The concentration of $O_2$ present in the gas is probably enough to convert all the produced carbon-11 hot-atoms into $^{11}CO_2$.

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Chapter III.

# THE PREPARATION OF CARBON-11 LABELLED α-AMINO ACIDS

## *III.1. INTRODUCTION.*

Radioactive organic compounds can be prepared by organic synthetic, biosynthetic or radiochemical methods or by exchange reactions (1). The organic synthetic approach is very often prefered, because the position of the radioactive label in the molecule can be controlled by the choice of the synthetic route. For the synthesis of physiological compounds which are difficult to prepare, for instance specific stereo isomers, a biosynthetic method is sometimes chosen. However, a lack of control over the yield and the position of the introduced label is a disadvantage. When each method is judged, the conclusion must be that they are not competitive but complementary. Radiochemical methods as recoil and radiolytic methods are not generally applicable. The low specific activity of the product and the complexity of the reaction mixture is a serious drawback for the preparation of complicated organic compounds. But for the preparation of small molecules (e.g. $^{11}CO_2$ and $H^{11}CN$) the methods can be used with success. In special cases exchange reactions, combined with a radiochemical method, are prefered to achieve the desired result (2).

In planning a synthesis of a labelled compound, a reaction scheme must be chosen that fulfils as far as possible the following requirements:

- the reaction in which the isotope is introduced into the molecule must give a high yield,
- the label must be introduced as nearly the last step of the synthesis as possible,
- the label must be introduced in a known position in the molecule,
- the product must be of a high radiochemical purity and of an adequate specific activity.

If a short-lived labelled compound must be prepared, still other requirements have to be satisfied (3). The short half-life of the radionuclide and the limitation to only a few starting materials are the most distinctive features which set the chemistry with short-lived isotopes apart from the "ordinary" chemistry with "classical" longer-lived isotopes. Arising from the nature of the research problems to which these short-lived isotopes are applied - the use as radiopharmaceuticals in Nuclear Medicine - the criterion has to be set that delivery of the product must be achieved within three times the half-life of the radionuclide, otherwise the radiochemical yield is too low to use the compound for diagnostic purposes. For carbon-11 work this means that only 60 minutes are available from the end of the radionuclide production to the moment the product is ready for administration to the patient.

The preparation of inorganic compounds labelled with short-lived cyclotron-produced isotopes is often a combination of the preparation of a radioactive precursor and of a synthetic method. To obtain an useful amount of high specific active product the activity range of the starting material is in the order of 100 mCi - 1 Ci.

Another point that has to be considered is the personal protection during the synthesis and purification of the product. It is absolutely necessary to carry out all the manipulations behind a lead shield, so a procedure that is easily to perform must be developed.

## *III.2. POTENTIAL USEFUL SYNTHESES FOR THE PREPARATION OF CARBON-11 LABELLED α-AMINO ACIDS.*

In principle two approaches exist for the synthesis of α-amino acids. One possibility is to introduce an amino group into a molecule that already contains the acidic function. The other approach is the synthesis by a condensation reaction with an aldehyde or by a condensation reaction with an aminomalonic ester in which the amino function, free or protected, is already present. In both cases DL-α-amino acids are obtained.

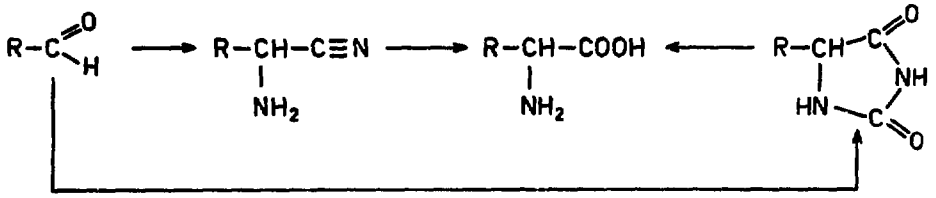
Using an amination reaction for the preparation of $^{11}$C-amino acids suffers from the disadvantage that the radionuclide must be introduced into the synthesis in too early a stage. While the criterion is set that a maximum of 60 minutes is available for the radioactive part of the synthesis and for the purification, we must

set aside this route.

Considering that at the moment only "on line" production methods for $^{11}CO$, $^{11}CO_2$, $H^{11}CN$ and $H^{11}C{\equiv}CH$ are reported in the literature (see II.5.), the following synthetic possibilities are left for the preparation of carbon-11 labelled α-amino acids.

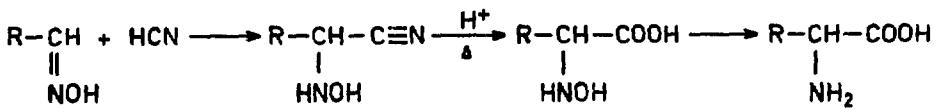
*Strecker synthesis.*

An α-amino nitril prepared by the reaction of ammonia and hydrocyanic acid with an aldehyde is hydrolysed to an amino acid (4). This method was modified by Bucherer (5) and by Bergs (6). They prepared a hydantoin by the reaction of NaCN and $(NH_4)_2CO_3$ with the bisulfite addition product of an aldehyde. The desired amino acid was obtained by hydrolysis of the hydantoin.



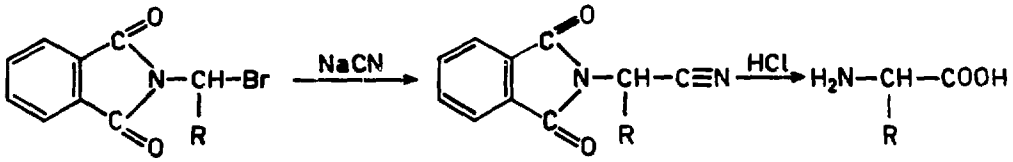
*Via α-hydroxyamino-carboxylic acids.*

α-Hydroxyamino-carboxylic acids are prepared by the addition of HCN to aldoximes. After hydrolysis and reduction α-amino acids are obtained (7, 8).



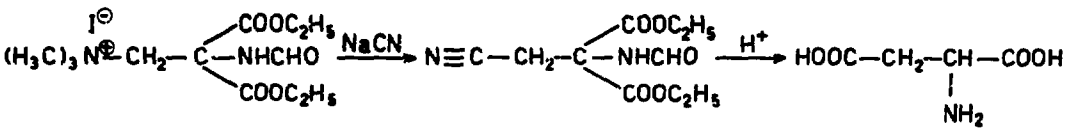
*Via N-bromoalkylphtalimides.*

Amino acids which form phtaloyl derivatives, e.g. glycine, alanine and leucine, can be prepared by the reaction between sodium cyanide and N-bromoalkylphtalimide (9). However the synthesis time reported in the literature is long (15 hours).




*Synthesis of DL-aspartic acid..*

DL-aspartic acid can be prepared by the reaction between NaCN and diethyl α-dimethylaminomethyl-α-formamidomalonate-methiodide followed by hydrolysis (10).




*Discussion.*

The good yields reported in the literature for the preparation of $^{14}C$-amino acids (11, 12) (although with synthesis times too long for carbon-11 work) and the preparation of $^{11}C$-lactic acid (13) by the reaction of $K^{11}CN$ and acetaldehyde encouraged us to evaluate the Strecker synthesis for the preparation of $^{11}C$-amino acids. In our hands however, within the time limit of 60 minutes, the maximum radiochemical yield we could afford for alanine was only 10 % (the synthesis was carried out with $K^{14}CN$). Besides this the synthesis

was difficult to reproduce. Another drawback of the method is that the hydrolysis must be carried out in a sealed ampoule. With highly radioactive material emitting γ-rays this is a risky manipulation. We have set aside this method.

Gelbard (14) claims success with the preparation of valine-$^{11}$C and alanine-$^{11}$C by the Strecker synthesis, however the only information he gives is that he is able to make them.

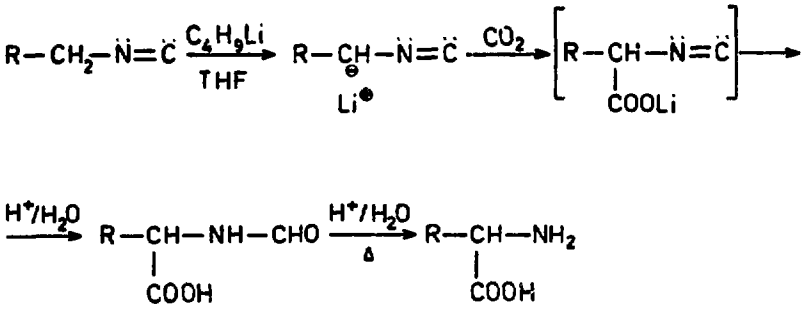
The synthesis time reported for the preparation via α-hydroxyamino-carboxylic acids and via N-bromoalkyl-phtalimides are too long for carbon-11 work. We did not evaluate these methods for the introduction of carbon-11 into amino acids.

The preparation of DL-aspartic acid-$^{11}$C by the method of Atkinson (10) was performed by Weimer (15). The achieved chemical yield within a total preparation time of 80 minutes is 30 %. The radiochemical yield is 1.9 %.

### *III.3. A NEW α-AMINO ACID SYNTHESIS.*

From III.2. can be concluded that only a few synthetic approaches are reported in the literature, which can perhaps be modified to prepare α-amino acids-1-$^{11}$C. In all the mentioned cases Na$^{11}$CN or H$^{11}$CN is the radioactive precursor. After the disappointing results obtained with the Strecker synthesis an investigation was started to develop an amino acid-1-$^{11}$C synthesis with $^{11}CO_2$ as radioactive precursor. The group of Schöllkopf reported

(16) that "α-isocyano-acetic or -propionic esters could be alkylated through their derivatives anionized at the α-position, to yield higher α-isocyano alkanoic esters that can be hydrolysed to amino acids". The preparation of α-lithioisocyanides in tetrahydrofuran (THF) solution was already described in 1968 by the same group (19). After considerable experimentation we developed a satisfactory method to prepare amino acids by a carboxylation. We treated some α-lithioisocyanides with $CO_2$ and hydrolysed the intermediate reaction products to amino acids.



By this method DL-α-phenylglycine (17) (yield 78 %) and DL-α-phenylalanine (yield 32 %) are prepared. Subsequently another group reported the same method (18). The products are obtained within 40 minutes after the introduction of $CO_2$ in the reaction sequence.

Our method is also used for the preparation of DL-α-phenylglycine-1-$^{11}$C and DL-α-phenylalanine-1-$^{11}$C.

*III.4. GENERAL PROCEDURE.*

Benzylisocyanide and phenylethylisocyanide were

prepared according a general procedure described by Ugi (20, 21). The method consists of the dehydration of N-monosubstituted formamides using phosgene or $POCl_3$ in the presence of tertiary amines. We also used the method of Appel (22). In that case the dehydration was achieved with triphenylphosphine as dehydrating agent in the presence of triethylamine.

The N-monosubstituted formamides were synthesised from the corresponding amines following the recommendations of Boudet (23) and Baumgarten (24).

$$R-NH_2 + HCOOH \underset{H_2O}{\overset{-H_2O}{\rightleftharpoons}} R-NH-CHO \underset{H_2O}{\overset{-H_2O}{\rightleftharpoons}} R-\dot{N}=\ddot{C}$$

To prevent polymerisation of the foul smelling isocyanides they were stored under nitrogen in a rubber stoppered bottle at $-20^{\circ}$.

The α-lithioisocyanides were prepared by the method of Schöllkopf and Gerhart (19). They carried out their syntheses in a nitrogen atmosphere and excluded water. We prefered the use of a vacuum system because such a system is more suitable for the preparation of radioactive compounds and is more convenient for manipulating small quantities of volatile material. After external cooling of the coloured α-lithioisocyanide solution in THF with liquid nitrogen a measured amount of carbon dioxide was condensed in the reaction vessel and after warming, the mixture was stirred for some minutes at $-65^{\circ}$, until the colour of the anion had disappeared.

The procedure was continued by adding an aqueous solution of HCl or HBr, warming the reaction mixture to room temperature and evaporating the solution under reduced pressure. To the residue another aliquot of mineral acid was added and the resulting suspension was hydrolysed by heating it under reflux for about 5 minutes. The obtained solution was filtered if necessary and evaporated again under reduced pressure. The residue was dissolved in aqueous ethanol. This solution was filtered and the filtrate neutralised with NaOH. The precipitate which formed was collected, dried under vacuum over $P_2O_5$ and identified as amino acid as described in the experimental part.

Schöllkopf and Gerhart (19) prepared their α-lithio-isocyanides by adding the isocyanide as THF-solution to a solution of n-butyllithium at a temperature of $-70^{\circ}$. They obtained an intense yellow-red solution in the case of α-lithiobenzylisocyanide. It is our experience, when we allow the reaction mixture to reach a temperature of $-50^{\circ}$ the colour of the solution turns to brown and the yield on the final α-phenylglycine increases from 65 % to 78 %. No increase in yield is observed for the preparation of phenylalanine, when the anion was formed at $-50^{\circ}$ instead of $-60^{\circ}$, although we observed a colour change of the solution from orange-red at $-60^{\circ}$ to red-brown at $-50^{\circ}$ (table III.4.1.).

We also varied the ratio of the amounts of n-butyl-lithium and isocyanide. No variation in the yield of the amino acid could be observed with increasing excess of

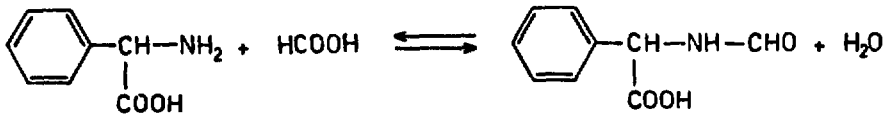
n-butyllithium (table III.4.1.).

| | mMole BuLi | Temp. of anion formation (°C) | Amino acid yield (%) |
|---|---|---|---|
| 5 mMole Benzyl-isocyanide | 5.0 | -70 | 65 |
| | 5.0 | -60 | 70 |
| | 5.0 | -50 | 78 |
| | 10.0 | -50 | 78 |
| | 20.0 | -50 | 76 |
| 3 mMole Phenylethyl-isocyanide | 3.0 | -60 | 26 |
| | 4.5 | -60 | 25 |
| | 6.0 | -60 | 32 |
| | 6.0 | -50 | 25 |

***Table III.4.1.*** **The effect of the ratio between n-butyllithium and isocyanide and the effect of the temperature of the anion formation on the amino acid yield.**

## *III.5. INTERMEDIATES AND SPEED OF HYDROLYSIS.*

To determine the intermediate reaction products in the synthesis of phenylglycine we tried to isolate α-isocyano-phenyl-acetic acid, however without success perhaps due to the instability of the isocyano group. We were more successful in the isolation of DL-N-formyl-phenyl-amino acetic acid. The compound was identified by comparison with a sample prepared by a method reported by Fischer (25).



Because the time scale is important in the synthesis of short-lived labelled compounds, we determined the time-yield optimum for the hydrolysis of DL-N-formyl-phenyl-amino acetic acid to DL-α-phenylglycine. This formamide was converted to the amino acid almost quantitatively within 5 minutes by heating in 10 % HCl under reflux.

## *III.6. RADIOACTIVE SYNTHESES*

### *DL-α-phenylglycine-1-$^{11}C$.*

DL-α-phenylglycine-1-$^{11}C$ was prepared by carboxylation of α-lithiobenzylisocyanide with $^{11}CO_2$. The syntheses were carried out at a 0.5 mMole scale of isocyanide. $CO_2$ (1 mMole) was added to the reaction mixture as carrier. We were able to reduce the elapsed time to 40 minutes between the end of the $^{11}CO_2$ production and the moment pure crystalline amino acid was obtained.

The chemical yields of the $^{11}C$-experiments calculated with respect to benzylisocyanide were between 35 % and 45 %. That in the experiments without radioactive material a higher yield was obtained was perhaps due to the facts that the mMole scale of the experiments with radioactive material is lower by a factor 10 and because a smaller excess of $CO_2$ was used.

The radiochemical yield calculated with respect to $^{11}CO_2$ (without decay correction) was in different experiments about 6 %. 25 mCi of $^{11}C$-labelled phenylglycine with a specific activity of 1 mCi/mg could easily be prepared.

Because the short half-life of carbon-11 makes an assay of the radiochemical purity of the end product by thin-layer chromatography and determination of the radioactive spots of the developed plate by counting the $^{11}C$ radioactivity cumbersome, in some experiments we added $^{14}CO_2$ to the reaction mixture. With different TLC-systems we could only detect one radioactive spot and this spot could be coloured with ninhydrin reagent. The spot had the same $R_f$ as an authentic sample of DL-α-phenylglycine (see figure III.6.1.). In the mother liquor besides uncrystallised DL-α-phenylglycine-1-$^{14}C$, we detected another radioactive compound which we did not identify (see figure III.6.1.).

The chemical identity and the purity of the end product was further checked by U.V. and I.R. spectra and by elemental analysis.

The nature of the radioactive label was controlled by determination of the half-life of the radionuclide and by γ-ray spectroscopy.

*DL-α-Phenylalanine-1-$^{11}C$.*

The synthesis of DL-α-phenylalanine-1-$^{11}C$ proceeds analogously to the DL-α-phenylglycine-1-$^{11}C$ synthesis. However the yield is lower. In the case of phenylglycine-

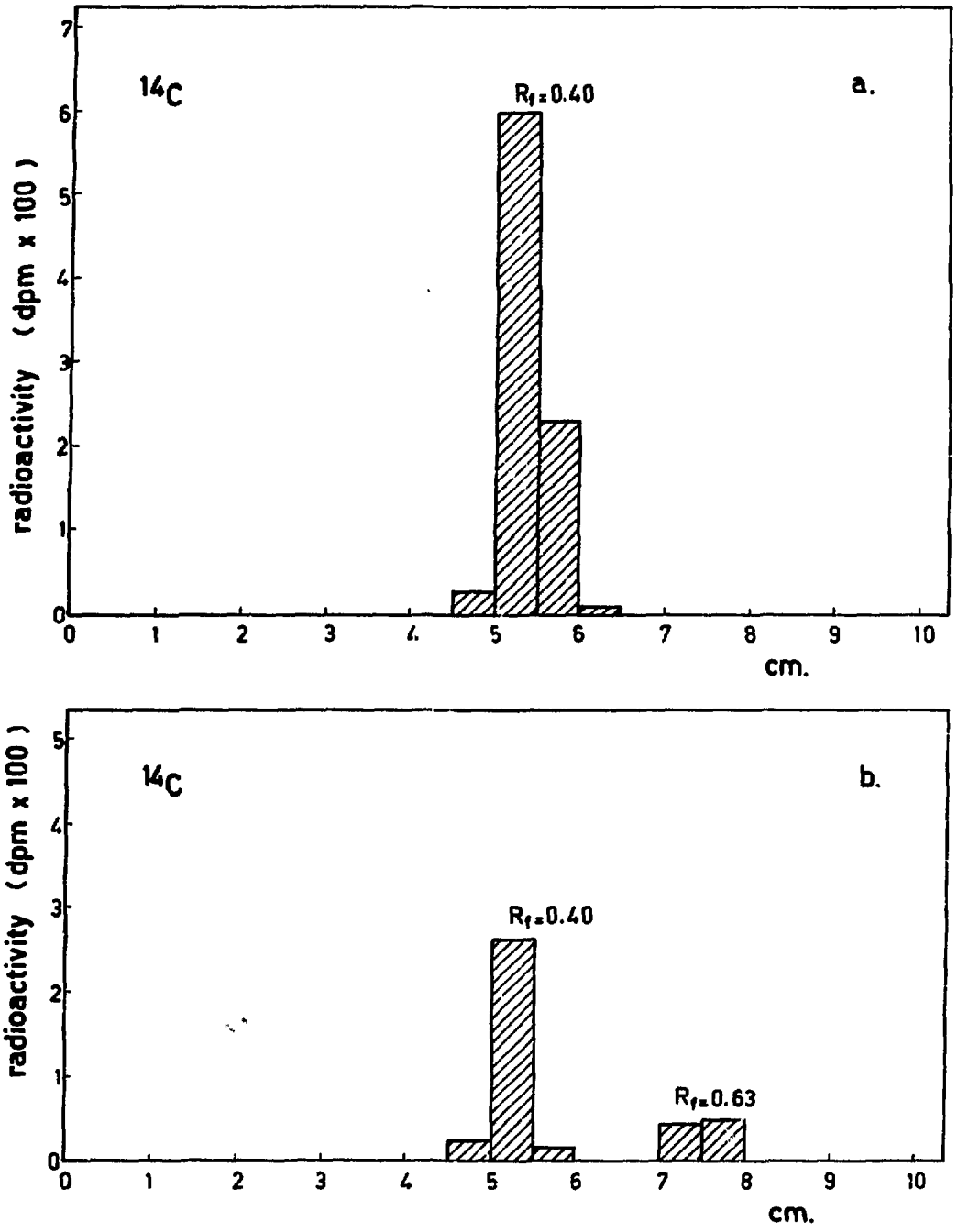



*Figure III.6.1.* Distribution of activity on thin-layer chromatograms (silicagel) of (a), DL-α-phenylglycine-1-$^{14}C$ and (b), of the mother liquor. Solvent: n-butanol-acetic acid-water (80 + 20 + 20 v/v).

1-$^{11}$C we obtained a light yellow product that can easily be purified. However the crude end product of the α-phenylalanine synthesis is a brown yellow oil. Normally the synthesis of phenylalanine was carried out with 3 or 4 mMole of phenylethylisocyanide. When the mMole scale was reduced to 0.5 we could obtain only occasionally pure product by crystallisation within the time limit set. The precipitation of the amino acid after neutralisation with alcoholic NaOH was very often retarded by the impurities. We tried to improve the purification method by dissolving the crude end product in acetic acid and to precipitate the phenylalanine with 1.4-dioxane. We also dissolved the impure material in absolute ethanol and neutralised the solution with an ethanol-aniline or ethanol-pyridine mixture. However non of these methods afforded us a more reliable purification method. Therefore, finally a method was adapted of using a small (12 x 0.5 cm) Dowex 50W x 8 ($H^+$) column for the preparation of phenylalanine-1-$^{11}$C. A disadvantage was that a longer time period (about 70 minutes) elapsed before the $^{11}$C-labelled phenylalanine was ready for animal experiments. Before the crude reaction product was brought onto the column the dark brown impurity formed during the hydrolysis was removed and the pH of the solution adjusted with NaOH to pH = 4.5. The impure amino acid was brought onto the column in aqueous solution. After washing the column with 20 ml of water the phenylalanine-1-$^{11}$C was eluted with 7 N $NH_4OH$. An elution curve is given in figure III.6.2. The water fractions contained

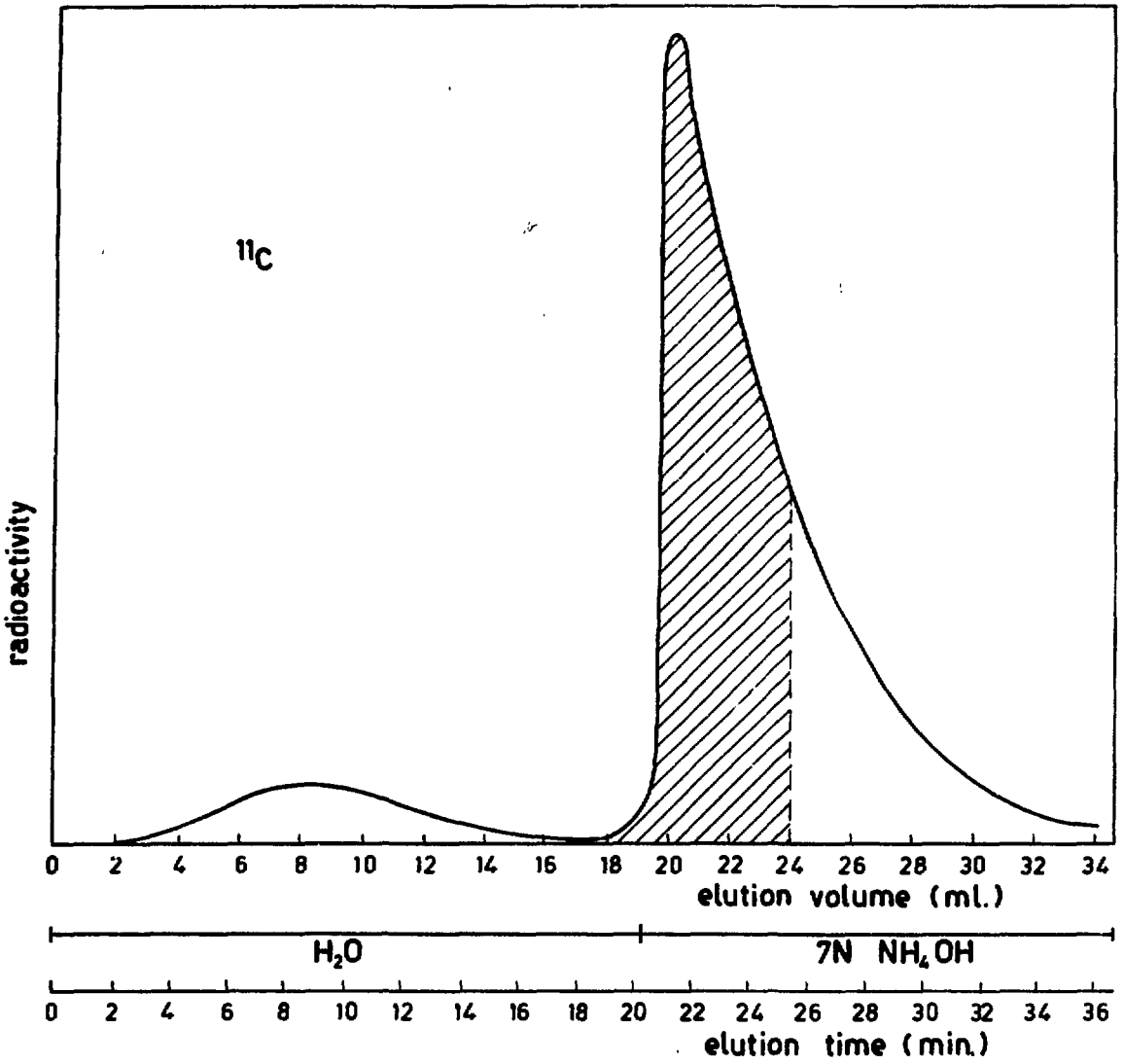



*Figure III.6.2.* The purification of DL-α-phenylalanine-1-$^{11}$C on Dowex 50W x 8 ($H^+$) 50 - 100 mesh. Typical chromatogram from radioactivity detection.

about 30 % of the radioactivity brought onto the column. The first 4 ml of the $NH_4OH$ eluate (shaded area of the elution curve) contained pure DL-α-phenylalanine-1-$^{11}$C. For animal experiments (see chapter IV.) these two

fractions were combined and made isotonic. In several experiments we obtained 4 mCi of DL-α-phenylalanine-1-$^{11}$C (radiochemical yield 1 %).

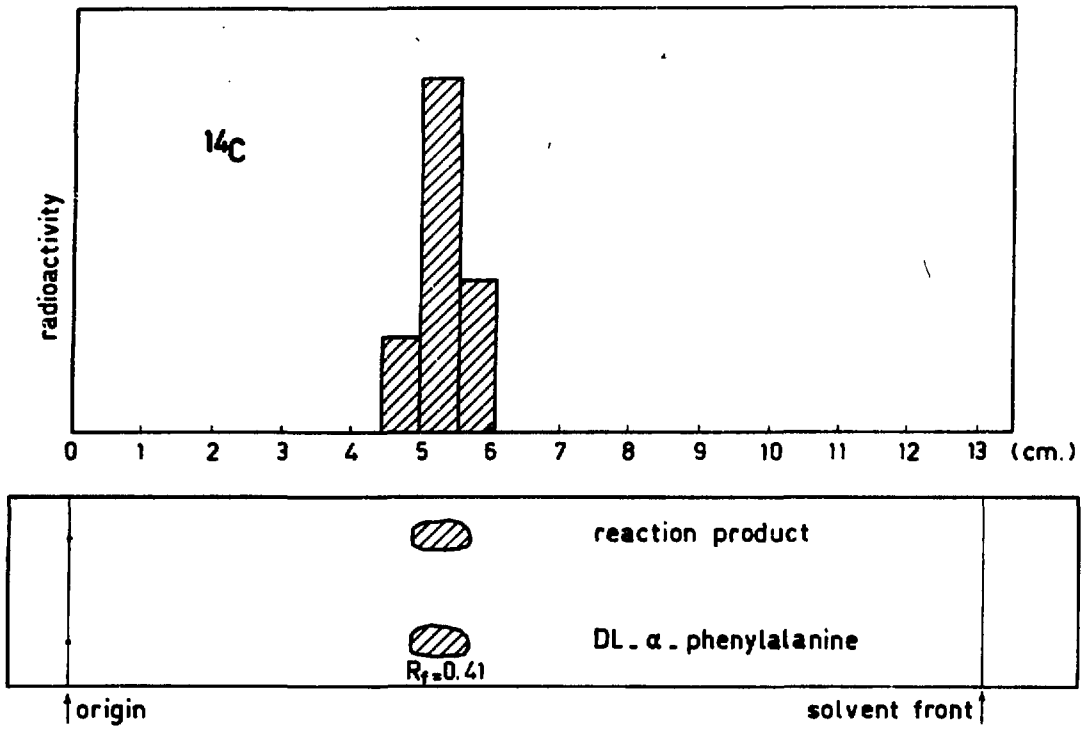



*Figure III.6.3.* Example of the chemical and radiochemical purity of DL-α-phenylalanine-1-$^{14}$C. Layers: silicagel. Solvent: n-butanol-25 % ammonia (75 + 25 v/v). Visualization with ninhydrin reagent. Radioactivity measurement by liquid scintillation counting.

The purity of the phenylalanine-1-$^{11}$C was determined after decay by thin-layer chromatography. Only one spot could be detected after colouring the thin-layer with ninhydrin reagent or with $I_2$-vapour. The spot in the different thin-layer systems used had the same $R_f$ as

DL-α-phenylalanine.

In the next $NH_4OH$ fractions of the column eluate an impurity was detected which could be coloured with ninhydrin reagent. On different thin-layer systems, this

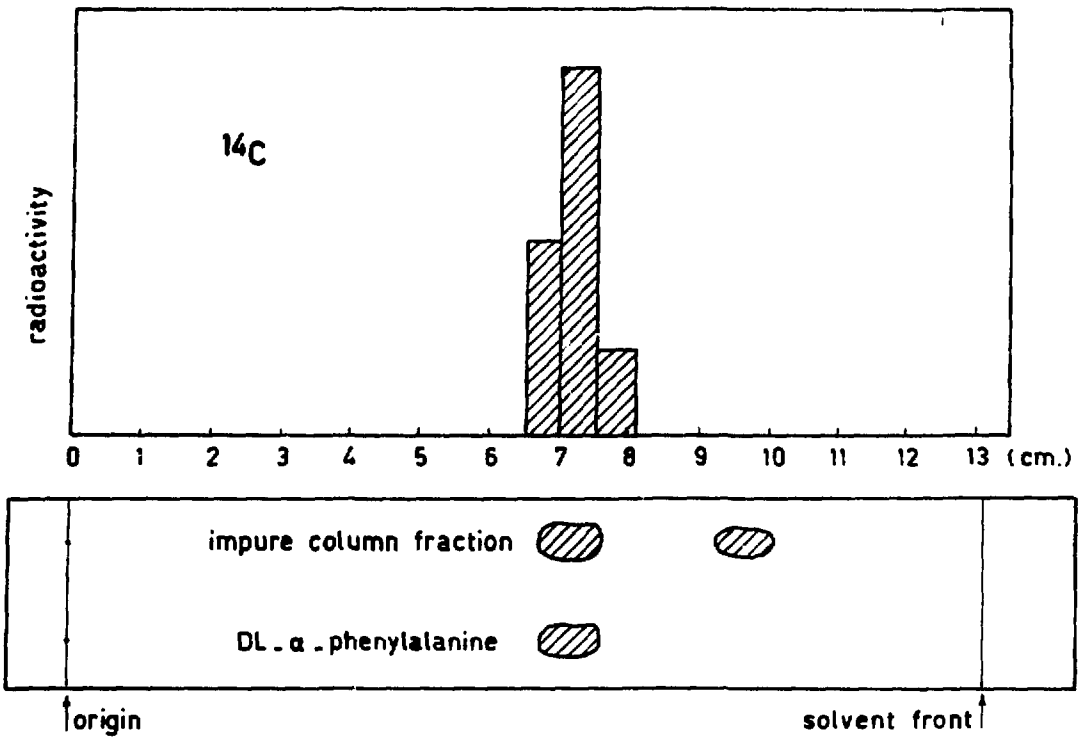



*Figure III.6.4.* Thin-layer chromatograms (silicagel) of an $NH_4OH$ column eluate fraction containing impure DL-α-phenylalanine-1-$^{14}C$. Solvent: n-butanol-acetic acid-water (80 + 20 + 20 v/v). Visualization with ninhydrin reagent. Radioactivity measurement by liquid scintillation counting.

impurity behaved identically with a sample of N-(β-phenylethyl)-formamide prepared by the method of Baumgarten (22).

In some experiments the carboxylation of the phenylethylisocyanide was carried out with $^{11}CO_2$ and $^{14}CO_2$.

A mixture of $^{11}C$-labelled and of $^{14}C$-labelled amino acid was obtained. After decay of the carbon-11 radioactivity, the radiochemical purity of the DL-α-phenylalanine-1-$^{14}C$ in the two first $NH_4OH$ fractions of the column eluate was determined. No radioactive impurity could be detected by thin-layer chromatography, combined with liquid scintillation counting (figure III.6.3.). The next fractions of the $NH_4OH$ eluate also appeared to be radiochemically but not chemically pure (figure III.6.4.).

## *III.7. EXPERIMENTAL PART.*

### *General remarks.*

Infrared spectra were taken on a Perkin Elmer 257 spectrophotometer. Ultraviolet spectra were recorded on a Zeiss PMQ II. Melting points were determined on a Mettler melting point apparatus and are uncorrected. Carbon-14 radioactivity was measured with a Nuclear Chicago Mark II liquid scintillation counter. Elemental analyses were carried out in the analytical department of the Chemical Laboratory.

The chemicals used were obtained from Merck, Darmstadt. The concentration of n-butyllithium in hexane (ca. 20 % solution) was determined by titration (26). The tetrahydrofuran (THF) used was distilled under low pressure from $LiAlH_4$ directly into the reaction flask.

As thin-layers were used silicagel (Alufolien Merck, Darmstadt). As solvents for thin-layer chromatography were used:

A. n-butanol-25 % ammonia (75 + 25 v/v)

B. n-butanol-acetic acid-water (80 + 20 + 20 v/v)

The thin-layers were coloured by the ninhydrin method (27) and by $I_2$-vapour (28).

Ninhydrin reagent: 0.2 gr ninhydrin dissolved in 100 ml of n-butanol saturated with water.

Treatment of TLC : heating at $110^{\circ}$ for 5 minutes.

$I_2$-vapour : the chromatograms were introduced in a closed vessel on the floor of which some crystals of iodine had been placed.

The carbon-14 radioactivity distribution on the thin-layer chromatograms were measured in the following way: the chromatograms were cut in pieces of 0.5 cm, each piece was introduced in a counting vial, a scintillation mixture was added and the radioactivity measured by liquid scintillation counting.

Scintillation mixture (29):

4 g of 2.5-diphenyloxazole (PPO),

0.2 g of 2.2'-p-phenylene-bis-(5-phenyloxazole) (POPOP),

60 g of naphtalene,

100 ml of methanol,

20 ml of ethylene glycol,

1.4-dioxane to make 1000 ml.

*DL-α-Phenylglycine.*

On a vacuum line 40 ml of tetrahydrofuran was distilled from $LiAlH_4$ into a 100 ml flask containing 5 mMole of n-butyllithium in hexane (ca. 2.5 ml of a ca. 2 N solution).

The mixture was cooled to -180° and a syringe was used to introduce 0.5 g of benzylisocyanide (4.27 mMole) into the reaction flask. The reaction mixture was allowed to reach a temperature of -50° and was stirred for 10 minutes. The colour changed from yellow to brown. The reaction flask was cooled again with liquid nitrogen to -180° and 50 mMole of $CO_2$ was condensed into the reaction mixture from a gasburette attached to the vacuum line. After the carboxylation had been completed by stirring the mixture for 5 minutes at -65°, 25 ml of 2 N HCl was added and the flask warmed to room temperature. The colourless or light yellow solution was evaporated under vacuum and to the white or light yellow residue 15 ml of 10 % HCl was added. The resulting suspension was heated under reflux for 5 minutes at atmospheric pressure. The clear light yellow reaction mixture was evaporated to dryness and the crude α-phenylglycine obtained dissolved in 40 ml of 60 % aqueous ethanol. The solution was filtered and the filtrate neutralised with 2 N NaOH. The precipitate which formed was collected, washed with absolute ethanol and dried under vacuum over $P_2O_5$. 503 mg (78 %) of a colourless product was obtained.

The identity of the material was determined by comparison of the I.R. and U.V. spectra with an authentic sample of DL-α-phenylglycine and by preparation of the methylesterhydrochloride. The I.R. spectrum and the melting point of the derivative were identical with those of a sample prepared in the same way from DL-α-phenylglycine. No suppression of the melting point was

observed by mixing the two. The purity of the amino acid was determined by thin-layer chromatography on silicagel with solvent systems A and B. Only one spot was observed with the same $R_f$ as DL-α-phenylglycine when the plate was coloured with $I_2$-vapour or ninhydrin reagent.

*DL-N-Formyl-phenyl-amino acetic acid.*

α-Lithiobenzylisocyanide, prepared from 0.5 g (4.27 mMole) of benzylisocyanide was carboxylated by the procedure described for the preparation of DL-α-phenylglycine. After 25 ml of HCl was added, the aqueous layer was extracted with diethylether. The combined fractions were dried over $Na_2SO_4$ and evaporated to dryness. The light yellow residue was dissolved in a mixture of equal amounts of $CHCl_3$ and ether. When the ether was distilled out of the solution a white precipitate (571 mg, 75 %) formed. After recrystallisation from hot water, DL-N-formyl-phenyl-amino acetic acid was obtained. Mp. 175° (lit. 176.5 - 180°) (25).

Analysis: calcd: C, 60.76; H, 5.28; N, 7.82.
found: C, 60.13; H, 5.16; N, 7.64.

The I.R. and N.M.R. spectra were identical with those of a sample prepared by the method of Fischer (25).

*DL-α-Phenylalanine.*

An orange red coloured solution of α-lithiophenylethyl-isocyanide in 40 ml of dry THF was prepared by stirring under vacuum 470 mg (3.6 mMole) of phenylethylisocyanide for 10 minutes at -60° with 7 mMole of n-butyllithium

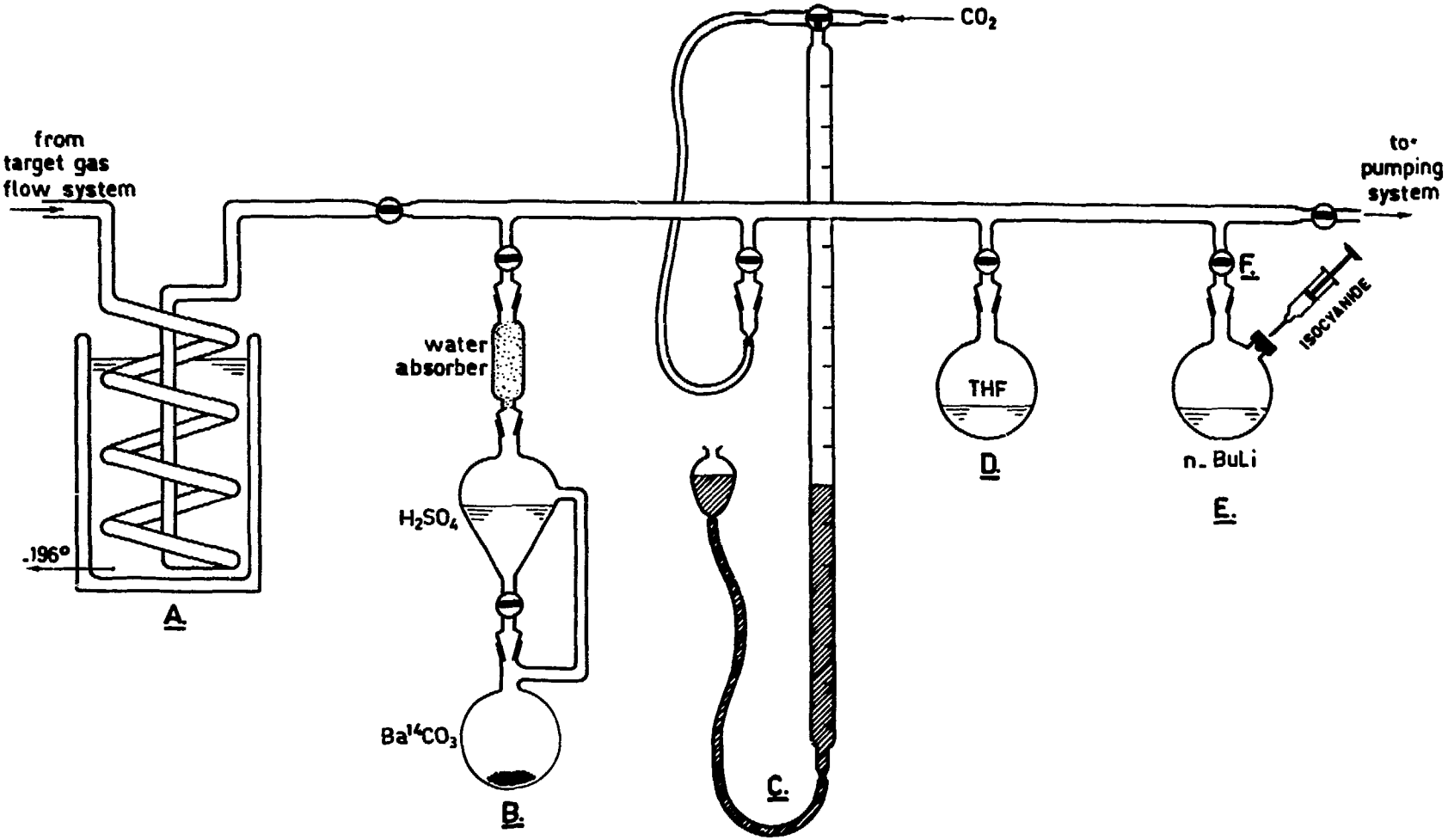



*Figure III.7.* Apparatus used for the preparation of $^{11}$C-amino acids.

(ca. 3.5 ml of a ca. 2 N solution in hexane). The lithio compound was carboxylated at -80° with 50 mMole of $CO_2$, the solution evaporated and the residue hydrolysed with 15 ml of 10 % HCl by boiling under reflux for 5 minutes. After evaporation a solid mixture with a yellow oil was obtained. The crude product was dissolved in 30 ml of 60 % aqueous ethanol and after neutralisation to pH = 7 with a 2 N solution of NaOH in 60 % aqueous ethanol, 191 mg (1.2 mMole, 32 % yield) of DL-α-phenylalanine was obtained. The identity of the material was determined by comparison of the I.R. and U.V. spectra with an authentic sample of DL-α-phenylalanine. The purity of the amino acid was determined by thin-layer chromatography on silicagel with solvent systems A and B. Only one spot could be detected by colouring the plate with ninhydrin or iodine vapour. The $R_f$ of the spot was the same as the $R_f$ of the authentic sample.

*General remarks on radioactive syntheses.*

The syntheses were carried out on a vacuum system shown in figure III.7. t = 0 is the moment the $^{11}CO_2$ collection from the bombarded target gas is finished (see II.8.). The time scheme is given in minutes.

*DL-α-Phenylglycine-1-$^{11}C$.*

In reaction flask E a solution of α-lithiobenzylisocyanide in 40 ml of dry THF was prepared from 60 mg (0.5 mMole) of benzylisocyanide and 0.5 mMole of n-butyllithium. The solution was chilled with liquid

nitrogen. From gasburette C 1 mMole of $CO_2$ was condensed into E.

The $^{11}CO_2$ was produced by proton bombardment of a gas flow of nitrogen mixed with 0.1 % $O_2$. The $^{11}CO_2$ was collected continuously from the gas flow for 30 minutes by a cold trap A cooled with liquid nitrogen.

t = 0 After collecting the $^{11}CO_2$, the cold trap A was evacuated. The $^{11}CO_2$ was distilled into reaction flask E by heating the trap to about + 100°. 2 ml of THF was distilled from vessel D into E to cover the $CO_2$ and $^{11}CO_2$. After the valve F had been closed, the mixture in E was stirred at -65° until the brown colour had disappeared. The cooling mixture was removed and 3 ml of 2 N HCl was added, followed by evaporation under vacuum. To accelerate the evaporation the flask was heated with a stream of hot air.

t = 18 To the solid residue 10 ml of 10 % HCl was added and the mixture was boiled until a clear solution was obtained.

t = 23 After another vacuum evaporation a light yellow solid was obtained.

t = 31 Flask E was removed from the vacuum line and the solid was dissolved in 10 ml of 60 % ethanol and the solution neutralised to pH = 7 with 2 N NaOH in 60 % aqueous ethanol.

t = 40 The formed precipitate was collected by filtration. Yield 28 mg (0.19 mMole, 37 % calculated with

respect to benzylisocyanide). Radiochemical yield 6 % (calculated on $^{11}CO_2$ without decay correction). The identity and purity of the material was controlled by I.R. and U.V. spectroscopy and by elemental analysis.

calcd: C, 63.57; H, 5.96; N, 9.27; O, 21.13

found: C, 63.06; H, 5.98; N, 9.03; O, 21.14

63.19; 6.06; 9,33; 20.97

*Radiochemical purity of DL-α-Phenylglycine-1-$^{11}C$.*

To assay the radiochemical purity of the prepared α-phenylglycine-1-$^{11}C$ in some experiments, besides the inactive carbon dioxide, $^{14}CO_2$ was introduced into the reaction mixture. In a $^{14}CO_2$-generator B (30). 5 μCi of $Ba^{14}CO_3$ (specific activity 61 mCi/mMole, Radiochemical Centre, Amersham, England) was converted with $H_2SO_4$ into $^{14}CO_2$ and the $^{14}CO_2$ was distilled into reaction flask E via an absorber filled with $CaSO_4$. After the carbon-11 had decayed, thin-layer chromatograms were made of the α-phenylglycine-1-$^{14}C$ and of the mother liquor. Solvent systems A and B were used. After development, the plate was cut in pieces of 0.5 cm and the radioactivity of each piece was determined by liquid scintillation counting. Only one radioactive spot was observed with the same $R_f$ as DL-α-phenylglycine.

*DL-α-Phenylalanine-1-$^{11}C$.*

In reaction flask E a solution of the desired anion in 10 ml of dry THF was prepared from 65 mg (0.5

mMole) of phenylethylisocyanide and 1.0 mMole of n-butyl-lithium. The solution was chilled with liquid nitrogen. From gasburette C 1.0 mMole of $CO_2$ was condensed into E.

t = 0 After collecting the $^{11}CO_2$ in A, the cold trap was evacuated at -180°. Then the $^{11}CO_2$ was distilled into E by heating A to about + 100°. To cover the $CO_2$ and $^{11}CO_2$, 2 ml of THF was distilled from flask D into E. After the valve F had been closed, the mixture in E was stirred at -65° until the orange red colour of the solution had disappeared. The cooling mixture was removed from the reaction flask and 3 ml of 2 N HCl was added, followed by evaporation under vacuum. To accelerate the evaporation the flask was heated.

t = 18 To the solid residue 4 ml of 5 N HCl was added and the mixture was boiled until a clear solution was obtained.

t = 23 After another vacuum evaporation a brown solid was obtained.

t = 31 Flask E was removed from the vacuum line and the solid was dissolved in 5 ml of absolute ethanol. To this solution 5 ml of water was added and the pH was adjusted to 4.5 with 1 N NaOH. The ethanol was boiled out of the solution. A tarry brown product precipitated on the wall of the reaction flask.

t = 38 The turbid colourless solution was brought onto a 12 x 0.5 cm column of Dowex 50W x 8 ($H^+$),

50 - 100 mesh. The column was washed with 20 ml of water.

t = 62 After washing the column the phenylalanine-1-$^{11}$C was eluted with 7 N $NH_4OH$. The radioactivity of the eluate was continuously monitored with a NaI-detector and a recorder. The $NH_4OH$ eluate was collected in fractions of 2 ml.

t = 66 The first two $NH_4OH$ fractions containing pure DL-α-phenylalanine-1-$^{11}$C, were combined (radiochemical yield 1 %).

*Chemical and radiochemical purity of DL-α-Phenylalanine-1-$^{11}$C.*

To determine the chemical and radiochemical purity of the prepared DL-α-phenylalanine-1-$^{11}$C, with some experiments, besides the inactive $CO_2$, some $^{14}CO_2$ was introduced into the reaction flask. The $^{14}CO_2$ was prepared as described for the determination of the radiochemical purity of phenylglycine-1-$^{11}$C. After the carbon-11 had decayed thin-layer chromatograms (silicagel) were made of the $NH_4OH$ solution using solvent system A and B. Only one spot with the same $R_f$ as an authentic sample of phenylalanine could be detected with $I_2$-vapour and with ninhydrin reagent. Thin-layer chromatograms, developed respectively with solvent systems A and B, were cut in pieces of 0.5 cm and the $^{14}$C-radioactivity of each piece was determined by liquid scintillation counting. On each thin-layer only one radioactive spot, with the same $R_f$ as DL-α-phenylalanine, was found.

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Chapter IV.

# DISTRIBUTION OF CARBON-11 LABELLED PHENYLALANINE AND PHENYLGLYCINE IN RATS

## *IV.1. INTRODUCTION.*

Unlike many other organs the pancreas is not amenable to X-ray examination. This makes the diagnosis of diseases affecting this organ rather difficult. Neoplasm of the pancreas has a very poor prognosis. Survival of patients with carcinoma of the pancreas for a period of 5 years after diagnosis of the disease is less than 5 percent (1).

During the last 10 years, considerable effort has been expended in developing radiopharmaceuticals for the visualisation of the pancreas by scintigraphy. However, at the moment, the only radioactive compound used for pancreas scintigraphy is selenomethionine-$^{75}$Se introduced in 1961 by Blau (2). The introduction of this radiopharmaceutical was based on the fact that the pancreas, an organ with a high rate of protein synthesis due to the production of digestive enzymes, has a large requirement for exogenous amino acids (3). Because it is difficult to label amino acids with externally detectable isotopes, Blau substituted $^{75}$Se for the sulphur atom of methionine. Although the pancreas has a high uptake of intravenously administered selenomethionine-$^{75}$Se this

radiopharmaceutical is not generally accepted in routine Nuclear Medicine practice. Disadvantages of this radioactive compound are a high liver uptake, a long effective half-life and unfavourable physical characteristics of the label. To overcome the disadvantages of $^{75}Se$, some $^{18}F$-labelled aromatic amino acids (4 - 7) were tested as pancreas scanning agents. Varma (8) investigated the pancreatic concentration of $^{125}I$-labelled phenylalanine in mice. However in most instances the biochemical behaviour of the labelled amino acids was different from the behaviour of the natural analogue. Lathrop (9) and Cohen (10) studied the organ distribution of $^{13}N$-labelled glutamic acid. Hara (11) injected L-aspartic acid-4-$^{11}C$ into mice to test the compound as tumour scanning agent. Weimer (12) investigated the organ distribution of this carbon-11 labelled amino acid in rats.

In this chapter the tissue distribution of DL-α-phenylalanine-1-$^{11}C$ and DL-α-phenylglycine-1-$^{11}C$ in rats is described and the uptake by the pancreas and the liver is compared with the accumulation of selenomethionine-$^{75}Se$ and with the accumulation of some $^{18}F$-labelled amino acids in these organs.

## *IV.2. EXPERIMENTAL.*

DL-α-phenylalanine-1-$^{11}C$ and DL-α-phenylglycine-1-$^{11}C$ were prepared as described in chapter III. The labelled amino acids were dissolved in diluted HCl, then the pH was adjusted to 5.5 with diluted NaOH. 0.2 ml of the

solution, containing about 1 mg of the amino acid, was injected into the femoral vein of male Wistar rats (160 - 200 g body weight) under light ether anaesthesia and the rats were killed after various time intervals. While each animal was still alive, but under ether anaesthesia, the abdomen was opened by a mid-line incision and blood was removed by aortic puncture. The radioactivity of the blood was determined by counting a 1 ml sample. After resection of the whole organ the radioactivity of the liver, spleen, kidneys and the pancreas was measured. An injection standard was measured under the same conditions. Finally the organs were weighed. Because of the difficulty encountered in the rapid removal of pure pancreatic tissue, this organ was excised with much fatty tissue according the procedure described by Scow (13) for total pancreatectomy in the rat. This procedure was adopted because we had to remove the organ as rapidly and completely as possible. Accurate figures for the weight of the pancreas in the rat were obtained in separate experiments by carefully resecting the pancreatic tissue. It was found that the weight of the pancreas obtained by the latter procedure was 0.4 % of the body weight.

## *IV.3. RESULTS AND DISCUSSION.*

Table IV.3.1. gives the concentration of radioactivity (± 1 S.D.) in liver, spleen, kidneys, pancreas and blood of the rats, 30, 45 and 70 minutes after intravenous injection of DL-α-phenylalanine-1-$^{11}$C and DL-α-phenylglycine

| Amino acid | Minutes after adm. | Number of rats | LIVER | | SPLEEN | | KIDNEYS | | PANCREAS | | BLOOD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % adm. dose | % adm. dose/g. | % adm. dose | % adm. dose/g. | % adm. dose | % adm. dose/g | % adm. dose | % adm. dose/g. | % adm. dose/ml. |
| DL-α-phenyl-alanine-1-$^{11}$C | 30 | 5 | 14.70 ± 1.58 | 2.24 ± 0.25 | 0.37 ± 0.11 | 1.04 ± 0.26 | 3.93 ± 0.57 | 2.40 ± 0.45 | 8.51 ± 1.79 | 11.62 ± 1.94 | 0.43 ± 0.05 |
| | 45 | 5 | 17.79 ± 1.01 | 2.40 ± 0.30 | 0.40 ± 0.10 | 1.06 ± 0.19 | 4.08 ± 0.52 | 2.43 ± 0.57 | 6.60 ± 1.20 | 9.80 ± 1.57 | 0.45 ± 0.10 |
| | 70 | 4 | 15.49 ± 1.54 | 2.11 ± 0.19 | 0.39 ± 0.07 | 1.07 ± 0.21 | 3.63 ± 0.66 | 2.11 ± 0.24 | 6.67 ± 1.58 | 9.07 ± 1.51 | 0.56 ± 0.08 |
| DL-α-phenyl-glycine-1-$^{11}$C | 30 | 7 | 3.42 ± 0.97 | 0.45 ± 0.07 | 0.21 ± 0.07 | 0.55 ± 0.13 | 3.55 ± 1.25 | 2.15 ± 0.54 | 2.18 ± 0.66 | 3.14 ± 1.03 | 0.49 ± 0.14 |
| | 45 | 5 | 2.42 ± 0.18 | 0.28 ± 0.05 | 0.19 ± 0.07 | 0.49 ± 0.19 | 1.91 ± 0.50 | 1.07 ± 0.10 | 1.87 ± 0.62 | 2.32 ± 0.92 | 0.35 ± 0.01 |
| | 70 | 5 | 1.83 ± 0.34 | 0.22 ± 0.05 | 0.17 ± 0.05 | 0.35 ± 0.10 | 1.68 ± 0.14 | 1.05 ± 0.07 | 1.23 ± 0.37 | 1.83 ± 0.35 | 0.24 ± 0.06 |

*Table IV.3.1.* Distribution of radioactivity in the rat after intravenous injection of $^{11}$C-amino acids.

1-$^{11}$C. The pancreas/liver concentration ratios (± 1 S.D.) for each of these amino acids are shown in table IV.3.2. In table IV.3.3. the pancreas concentration and pancreas to liver ratio of the labelled amino acids 30 minutes after injection are shown.

| | Minutes after injection | | |
|---|---|---|---|
| | 30 | 45 | 70 |
| DL-phenylalanine-$^{11}$C | 5.19 ± 1.04 | 4.08 ± 0.82 | 4.30 ± 0.81 |
| DL-phenylglycine-$^{11}$C | 6.98 ± 2.53 | 8.29 ± 3.61 | 8.32 ± 2.47 |

*Table IV.3.2.* Pancreas to liver ratio of $^{11}$C-labelled amino acids in rats.

The success of a radiopharmaceutical for pancreas scintigraphy depends on the percentage of the administered dose accumulating in the pancreas and of the ratio between the concentration of radioactivity per gram tissue of pancreas and liver.

The data in table IV.3.1. show that the amount of radioactivity concentrated in the pancreas after administration of DL-phenylglycine-$^{11}$C is considerably lower than after administration of DL-phenylalanine-$^{11}$C. The concentration of both amino acids in this organ does not change considerably between 30 and 70 minutes after

injection. The pancreas uptake of $^{11}C$-labelled phenylalanine 30 minutes after injection is about 3 times higher than the uptake of $^{18}F$-labelled fluorophenylalanine (table IV.3.3.). This suggests that in rats DL-fluorophenylalanines are less readily incorporated in cells than DL-phenylalanine.

| Amino acid | % adm. dose | % adm. dose/g. | Ratio pancreas liver concentr. | Ref. |
|---|---|---|---|---|
| DL-phenylalanine-$^{11}C$ | 8.5 | 11.6 | 5.2 | |
| DL-phenylglycine-$^{11}C$ | 2.2 | 3.1 | 7.0 | |
| DL-O-fluorophenylalanine-$^{18}F$ | 3.0 | 2.9 | 5.2 | (4) |
| DL-6-fluorotryptophan-$^{18}F$ | 8.7 | ± 9 | 9.1 | (7) |
| L-selenomethionine-$^{75}Se$ | 5.5 | 6.3 | 1.7 | (14) |

*Table IV.3.3.* Comparison of several amino acids for pancreas scintigraphy 30 minutes after injection in rats.

The uptake of phenylglycine in the pancreas after 30 minutes is of the same order as the uptake of fluorophenylalanines. From our figures for phenylalanine-$^{11}C$ and from the figures of Atkins (7) for 6-fluorotryptophan-$^{18}F$ it can be concluded that the same percentages of these amino acids injected into the rat reaches the pancreas. The uptake percentage for selenomethionine-$^{75}Se$ is lower.

The pancreas to liver ratio for DL-α-phenylalanine-$^{11}C$ is higher than the ratio for L-selenomethionine-$^{75}Se$. If

this is also true in man a better visualisation of the pancreas (possibly with a double isotope technique for subtracting liver activity) might be possible with DL-α-phenylalanine-1-$^{11}$C instead of L-selenomethionine-$^{75}$Se as radiopharmaceutical. This conclusion is the more true for 6-fluorotryptophan-$^{18}$F because the pancreas to liver ratio is higher than for phenylalanine-$^{11}$C. Because the $^{18}$F-analogue of aromatic amino acids is less readily incorporated in the pancreas than the $^{11}$C-analogue as is true for phenylalanine. Tryptophan-$^{11}$C would probably be an even better radiopharmaceutical for pancreas scintigraphy than 6-fluorotryptophan-$^{18}$F.

Another application of $^{11}$C-amino acids might be the *in vivo* measurement of protein turn over. This will perhaps open a door to the *in vivo* differentiation between types of tumours by measuring the amino acid turn over of tumour tissue.

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*SUMMARY*

Short-lived radionuclides ($t_{\frac{1}{2}}$ < 15 hr) have distinct advantages as labels for radiopharmaceuticals in Nuclear Medicine. The reduced radiation dose, to which the patient is subjected is perhaps the most important advantage.

Radionuclides of short half-life can be prepared in various ways but transport from source to patient is a limiting factor. Transport over long distances is only possible if a suitable parent-short-lived daughter generator system is available.

Until recently even simple organic compounds were not available as radiopharmaceuticals containing short-lived radionuclides. A recent development is the preparation of organic radiopharmaceuticals labelled with cyclotron-produced radionuclides of short half-life, whose radiation is measurable outside the patient's body. Examples are $^{11}C$ ($t_{\frac{1}{2}}$ = 20.4 min), $^{13}N$ ($t_{\frac{1}{2}}$ = 10 min), $^{18}F$ ($t_{\frac{1}{2}}$ = 110 min) and $^{123}I$ ($t_{\frac{1}{2}}$ = 13.3 hr). The preparation and use of these organic radiopharmaceuticals must be seen as a multi-step process requiring an interdisciplinary approach. Three of the most important steps are:

- the cyclotron production of a radioactive precursor
- the preparation of a radiopharmaceutical from this precursor
- administration of the radiopharmaceutical to the patient and the subsequent scintigraphic examination of specific areas of the patient's body.

The short half-life makes particular demands on the whole procedure. Since an upper limit of only three half-lives of the radionuclide is available for the entire multi-step procedure, high demands are made on the scientific, technical and organisational skill of the entire team.

Obviously a major advantage of this rapid decay lies in the possibility of readministration of the radio-pharmaceutical and re-examination of the patient on the same day.

The occurrence of the element carbon in nearly every biological compound, the short half-life and the nuclear properties of carbon-11 make the latter one of the most useful radionuclides in Nuclear Medicine.

The aim of this investigation was the preparation of some carbon-11 labelled amino acids and to test these compounds as radiopharmaceuticals for pancreas scintigraphy. Therefore we developed a new, rapid amino acid synthesis based on the carboxylation of α-lithio-isocyanides with $^{11}CO_2$, followed by hydrolysis of the intermediate reaction product to the desired amino acid. By this method DL-α-phenylalanine-1-$^{11}$C was obtained within 66 minutes and DL-α-phenylglycine-1-$^{11}$C within 40 minutes. The chemical yields calculated with respect to the isocyanides were respectively 32 % and 78 %.

The $^{11}CO_2$ used for the syntheses was prepared by a cyclotron with a yield of 1.5 mCi/μA.min via the nuclear reaction $^{14}N$ (p. α) $^{11}C$. A flow of nitrogen gas (mixed with 0.1 % oxygen) was bombarded with 20 MeV protons at a target gas pressure of 3 atmospheres. The construction

of the gas flow target system is described.

The carbon-11 labelled amino acids were administered intravenously to rats and the distribution over pancreas, liver, spleen, kidneys and blood was measured after several time intervals. From these results the ratio of the concentration in pancreas and liver was calculated and compared with the corresponding figures from the literature for some $^{18}F$-labelled aromatic amino acids and with the data for L-selenomethionine-$^{75}Se$. The results point out that DL-α-phenylalanine-1-$^{11}C$ is better suited and DL-α-phenylglycine-1-$^{11}C$ is less well suited to pancreas scintigraphy than L-selenomethionine-$^{75}Se$. However from the data in the literature and from our results we conclude that DL-6-fluorotryptophan-$^{18}F$ is perhaps more suitable for visualisation of the pancreas than DL-α-phenylalanine-1-$^{11}C$. The percentage of the administered dose accumulating in the pancreas for both amino acids is the same but the pancreas to liver ratio for DL-6-fluorotryptophan-$^{18}F$ is higher than for DL-α-phenylalanine-1-$^{11}C$.

This investigation indicates that the rapid synthesis of organic compounds containing short-lived radionuclides is feasable and that further developments in the synthesis of organ specific organic radiopharmaceuticals can be expected in the future.