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Review Article

Compounds Labelled with Short-Lived β^+-Emitting Radionuclides and Some Applications in Life Sciences. The Importance of Time as a Parameter

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Dedicated to Professor Göran Bergson on the occasion of his 65th birthday

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Some examples of recent development of the synthesis of compounds labelled with short-lived β^+-emitting radionuclides will be discussed with an emphasis on the importance of time in selecting a synthetic strategy. Furthermore the use of such labelled compounds to monitor certain processes in areas within the field of analytical chemistry and in various applications in drug development will be presented.

The application of radiotracer technology in life science and medicine began some eighty years ago with de Hevesey's pioneering work with the naturally occurring radionuclides ^{210}Pb and ^{212}Pb. In 1923 he used these as radiotracers in plant studies,[1] and in 1927 the first human study was performed using ^{212}Bi in patients with heart disease.[2] Later, from the development of accelerators and nuclear reactors came the increased availability and use of radionuclides such as the neutron-deficient ^{11}C or the neutron-rich ^{3}H and ^{14}C. The latter two especially were then extensively used in biology and chemistry.[3]

In the search for radiotracers of greater clinical value the accelerator-produced short-lived β^+-emitting radionuclides ^{11}C, ^{15}O, ^{13}N and ^{18}F came into focus with the development of suitable detector systems. During the last 20 years the *in vivo* application of molecules labelled with these nuclides has expanded significantly. Positron emission decay, resulting in two anti-parallel gamma photons (511 keV), gives special possibilities for localising the radiation source within a living organism by external detection. This has created a new dimension of radiotracer applications in the life sciences. In recent decades improvement in detector systems and development of reconstruction algorithms for image production has enabled the Positron Emission Tomography (PET) technique to become not only an important tool in biomedical research but also a clinically useful modality.[4] The sensitivity of the technique and the possibility of performing non-invasive studies with the short-lived β^+-emitting nuclides have thus opened up new ways of studying *in vivo* biochemistry and pharmacology in research animals and man.

A limitation on the further progress of tracer technology has been the access to labelled molecules suitable for biological applications. In order to address a given biological, pharmacological and medical question the following features need special consideration in the design of labelled tracer molecules: (i) The position of the label must be considered in relation to the metabolic pathway of the compound. (ii) The physical half-life of the radionuclide should be matched with the biological

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half-life of the studied process. The first point is exemplified with ^{11}C-labelled L-DOPA, where different tissue kinetics and PET images are obtained using tracers labelled either in the 1 or the 3 positions, respectively.[5] The second point is exemplified by the selection of ^{15}O ($t_{1/2}$ 2.03 min) or ^{18}F ($t_{1/2}$ 110 min). In repeated blood flow measurements, ^{15}O is ideal using ^{15}O-water, while ^{18}F, bound to the appropriate tracer, is preferable in studies of slower processes like the kinetics of protein synthesis, cell proliferation, etc.[4]

The selection of tracers might also be influenced by the following questions: (i) Are non-specific and specific processes discriminated? (ii) Are the studies of the acute or chronic type? (iii) Is it possible to visualise a specific biological function by the combination of tracers?

The development of methods and techniques for tracer synthesis is thus a key issue. Of importance was the recognition of time as a parameter in the same category as chemical yield and purity. The significance of time in synthesis using short-lived radionuclides was dealt with in one of the initial works on ^{11}C-labelling synthesis, where time and the concentrations of reactants were related to kinetic data.[6]

Increased access to labelled precursors, available for routine preparation, is one important feature in the development of labelling synthesis. Other aspects to consider are related to the production of short-lived radionuclides with high specific radioactivity, allowing studies of high-affinity receptors present in very low concentrations.

Inclusion of the radionuclide label late in the synthetic sequence is a final aspect to consider when evaluating synthetic strategies. The strategy is important for several reasons. Minimising the synthesis time will increase both the radiochemical yield and the specific radioactivity. These points will be discussed in this article, and the focus will be on recent progress in synthesis and applications in analytical chemistry and in drug development.

Radionuclide production

The most frequently used short-lived β^+-emitters are presented in Table 1, and some are important elements in the life sciences (^{11}C, ^{13}N and ^{15}O). These accelerator-produced radionuclides are obtained with high specific radioactivity using low-energy particle reactions, typically 8–20 MeV for H^+ or 5–15 MeV for $^2H^+$,[4] with gas, liquid or solid targets. The recovery of radioactivity as labelled precursors from the targets has been developed to the extent that it can be achieved in an automated fashion.

Precursor production

The development of new precursors[7] is important for the labelling of new types of substances and for increasing the number of potential labelling positions. A number of precursors which are available within a half-life from ^{11}C-carbon dioxide, obtained in the target at high specific radioactivity, are shown in Fig. 1.

The most frequently employed precursor is [^{11}C]methyl iodide.[8] This is usually prepared by converting [^{11}C]-carbon dioxide into [^{11}C]methoxide, followed by solvent removal and reaction with hydroiodic acid. It has been a useful alkylating agent for carbanions and nucleophilic hetero groups and is also used for preparation of several other valuable precursors such as [^{11}C]methyllithium,[9] [^{11}C]methylcuprates,[10] [^{11}C]nitromethane[11] and [^{11}C]methyltriphenylphosphorane.[12]

Hydrogen [^{11}C]cyanide,[13] obtained on-line from [^{11}C]carbon dioxide or [^{11}C]methane, is used in the synthesis of nitriles which can be converted into amines,

Table 1. β^+-Emitting radionuclides.

Radionuclide	Half-life	Nuclear reaction	Theoretical S.A.[a] (GBq/μmol)	Practical S.A.[a] (GBq/μmol)
^{11}C	20 min	^{14}N(p,α)^{11}C	3.4×10^5	10–200
^{15}O	2 min	^{14}N(d,p)^{15}O	3.4×10^6	–
^{18}F	110 min	^{18}O(p,n)^{18}F	6.3×10^4	50–400
^{76}Br	16 h	^{76}Se(p,n)^{76}Br	7.1×10^3	50–200

[a]S.A., Specific radioactivity defined as radioactivity per unit mass.

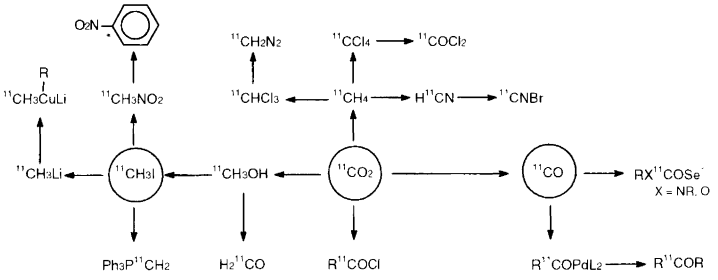


Fig. 1. Some examples of labelled precursors starting from $^{11}CO_2$.

amides and carboxylic acids. [^{11}C]Cyanogen bromide, rapidly prepared on-line from hydrogen [^{11}C]cyanide, is useful for labelling of macromolecules and nitrogen containing compounds such as guanidines.[14]

[^{11}C]Carbon monoxide is a versatile precursor for synthesis of labelled carbonyl compounds. Some of these labelled carbonyl compounds (i.e. aldehydes and ketones) are by themselves valuable precursors. It is likely that in a few years [^{11}C]carbon monoxide will reach the same importance as [^{11}C]methyl iodide in the routine production of PET tracers.

The short half-life of ^{15}O and ^{13}N has limited their usefulness for labelling purposes. Although ^{15}O-labelled water, oxygen gas, carbon monoxide, carbon dioxide and ^{13}N-labelled ammonia are used on a routine basis in medical applications they have not, with the exception of labelled ammonia, been used for synthetic applications.

Synthetic strategy

Several aspects, apart from those present in conventional synthesis, have to be regarded when planning syntheses of compounds labelled with short-lived β^+-emitting nuclides. For example, the time factor, radiation protection, labelling position and specific radioactivity are key factors worthy of careful consideration. In the production of tracers for *in vivo* application the final product has to be sterile, pyrogen-free and dissolved in a physiological vehicle. This has to be achieved within three half-lives of the radionuclide. As a consequence of the time constraint synthetic methods are often modified when used in tracer production. For example, drastic reaction conditions may be used in labelling procedures, despite the fact that the chemical yield is lower, if the increase in reaction rate is large enough. Time optimisation also influences the choice of protective groups and the type of procedure used for synthesis and work-up. Examples are the use of one-pot procedures,[15] ultrasound[16] and microwave technology,[17] in order to reduce production time by simplifying the technical handling and/or increasing reaction rates. The most important factor in rapid tracer productions is the small amount of labelled substance. This can be utilised to reduce the time of reactions, transfers and purifications.

Owing to the small amounts of labelled reagents, the stoichiometrical ratio between substrate and labelled reagent may be in the order 10^4 to 1. A result of this is that the labelled reagent is consumed faster because of pseudo-first-order reaction kinetics. Using such small amounts of substance is also beneficial for the technical handling. This is exemplified by the convenient application of semi-preparative HPLC and the possibilities of miniaturisation in order to facilitate automation and to speed up handling. It is relevant to state that the work on increasing specific radioactivity and the advent of new precursors and synthetic methods are very much related to technological development. The use of [^{11}C]carbon monoxide was possible owing to technical developments made in the application of supercritical ammonia in ^{11}C-labelling synthesis.[18]

The factors discussed above, combined with aspects on radiation safety, have pushed the need for development of synthetic technology that can meet the demands of routine pharmaceutical production. Therefore processor-controlled automated synthetic devices have been developed[19] and are routinely applied. This technology is furthermore mandatory in order to meet the demands related to Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP).[20]

Synthesis of compounds labelled with ^{11}C

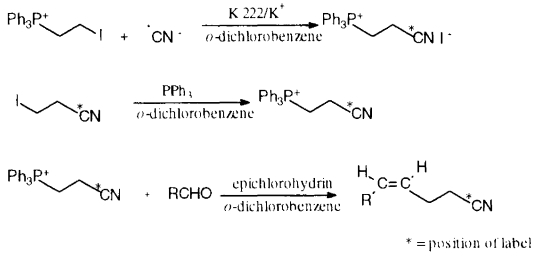
Alkylation reactions on N, O and S nucleophiles using ^{11}C-labelled methyl iodide. The first application of [^{11}C]methyl iodide was a synthesis of ^{11}C-L-methionine.[21] Later the utilization of ^{11}C-labelled methyl iodide in alkylations with N or O nucleophiles such as amines, amides, phenolates or carboxylates became the most common way for introducing ^{11}C into a molecule. A large number of receptor ligands and enzyme substrates have been ^{11}C-labelled using N or O nucleophiles.[7]

^{11}C–C bond-forming reactions. Although a substantial number of the compounds used as pharmaceuticals today contain an *N*-methyl group, and may thus potentially be labelled by [^{11}C]alkylating agents, this is not always the preferred position owing to metabolic cleavage. The need for synthetic strategies that give access to other labelling positions is obvious. The ability to build up key structural units for use in further coupling reactions is also important.

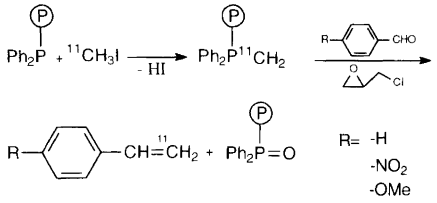
In tracer synthesis the following ^{11}C–C bond-forming reactions have been applied: (i) alkylation on a stabilised carbanion using a ^{11}C-labelled alkyl iodide[22] (e.g. asymmetric synthesis of ^{11}C-labelled amino acids); (ii) carbonation of an organometallic reagent with [^{11}C]carbon dioxide[23] (e.g. carboxyl ^{11}C-labelled fatty acids); (iii) reactions of [^{11}C]cyanide with electrophilic carbons[17] (e.g. [1-^{11}C]glucoseamine); (iv) reactions with the anion of a ^{11}C-labelled nitroalkane[11] (e.g. [1-^{11}C]norepinephrine); (v) cuprate-mediated coupling reactions using a ^{11}C-labelled alkyl iodide[24] or a ^{11}C-labelled methyl cuprate[10] (e.g. methyl ^{11}C-labelled fatty acids, ^{11}C-labelled steroids); (vi) alkene synthesis using [^{11}C]methylenetriphenylphosphorane[12] (e.g. ^{11}C-labelled monosaccharides).

Recently, palladium has found an increased use in ^{11}C–C bond-forming reactions. Aromatic cyanation, the Stille and Suzuki cross-coupling reactions and the Heck reaction are applied in a broad range of ^{11}C-labelling reactions. Of particular importance are the palladium mediated carbonylation reactions using [^{11}C]carbon monoxide, which are opening up new labelling pathways.

Synthesis and selective oxidation of ^{11}C-labelled alkenes. Terminal alkenes, labelled in the terminal methylene



Scheme 1.



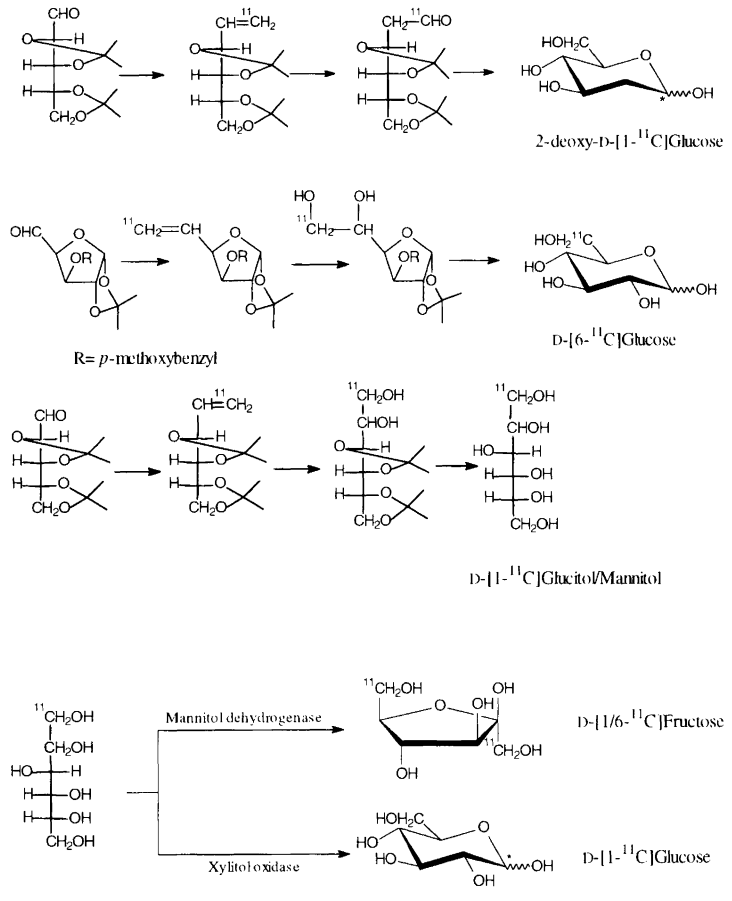
Scheme 2.

group, have been synthesised from [^{11}C]methyl iodide in a Wittig reaction.[12] Internal ^{11}C-labelled alkenes have been synthesised from [^{11}C]cyanoalkyl halides and [^{11}C]cyanoalkylphosphoranes (Scheme 1).[25] A method was developed to avoid the strong bases commonly used for the proton abstraction of the phosphorous ylide. The use of epichlorohydrin (1-chloro-2,3-epoxypropane) as a base precursor to generate an equimolar amount of base *in situ*[26] allowed base-sensitive compounds to be labelled with the Wittig reaction.

The use of polymer-bound triphenylphosphine in the synthesis of ^{11}C-labelled alkenes was evaluated in model reactions with three aromatic aldehydes (Scheme 2). This change improved the synthesis with respect to the number of manipulations and the total synthesis time, but the radiochemical yields were lower.

Selective oxidation of labelled alkenes provides a route to ^{11}C-labelled monosaccharides (Scheme 3).[27] Labelled alkenes were oxidised with osmium tetraoxide or palladium(II) chloride followed by removal of the protecting groups and, in some cases, enzymatic oxidation of the labelled polyol.

Enzyme reactions. Enzyme catalysis has for decades been used in labelling synthesis and shown to be an efficient approach to obtain high chemo-, regio- and stereoselectivity. The applications in labelling synthesis with short-lived radionuclides are particularly rewarding, since the small amounts of labelled substance make it possibility to achieve high yields and short reaction times with



Scheme 3.

low enzyme concentrations. Enzyme catalysis may thus give access to compounds that otherwise have been difficult to prepare within the time frame set by the used radionuclides.

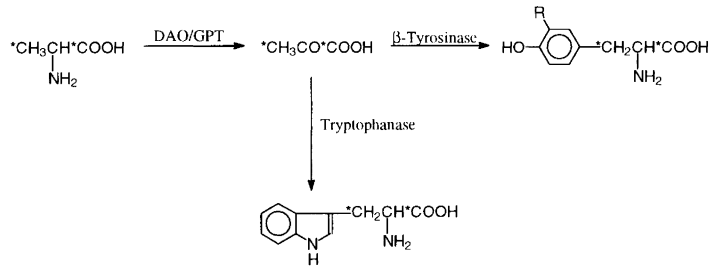
Amino acids constitute an important class of compounds in the body, where their functions include: building blocks for proteins, neurotransmitters or precursors for neurotransmitters. Amino acids labelled with short-lived positron emitting radionuclides have thus a great potential as tracers. Several amino acids have been labelled with ^{11}C, ^{13}N and ^{18}F, where one or more steps have been enzyme catalysed. Enzymes may have two functions in amino acid synthesis: (i) as resolving agents where either the enantiomers is converted to another compound or the desired enantiomer is obtained by selective hydrolysis of a protecting group; (ii) to create new bonds, e.g. C–N or C–C. Since the first approach means that half of the radioactivity is lost, the second approach has the potential of producing higher radiochemical yields. The main objective for using enzymes in labelling synthesis has been the challenge to develop reliable methods for ^{11}C-labelling of important amino acids. In several of these methods, the same labelled precursors and similar reaction sequences are used. This simplifies automation, which is important when high levels of radioactivity are used.

Enzymes are either used in the free form or immobilised on a solid support. The use of immobilised enzymes has several advantages. It reduces the risk of contaminating the product with trace amounts of biological material and gives the possibility to perform several syntheses with the same enzymes, which is beneficial from an economical point of view. It may also facilitate automation, which is important in the context of GMP and quality assurance mandatory when the product is used in humans.

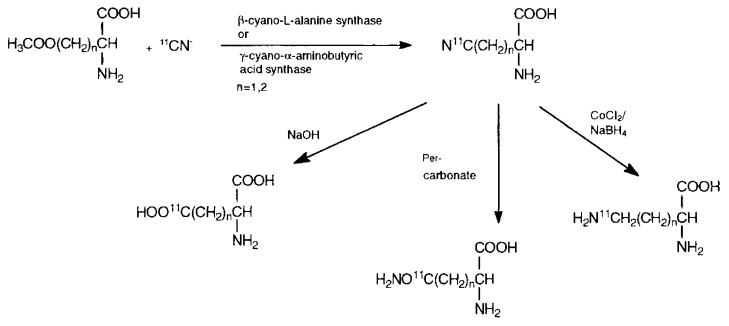
Enzyme-catalysed amino acid syntheses. The type of reactions covered here exemplify both approaches described above, i.e. creating new C–C bonds or transformations such as oxidations and reductions. Some of these methods give the possibility to label the amino acid in alternative positions. Labelling of the carboxylic position or another position of L-DOPA or L-5-hydroxytryptaphan is valuable tool to determine if it is possible to measure *in vivo* the rate of neurotransmitter synthesis (e.g. dopamine and serotonin).

An example of a combined chemical and enzymatic reaction route that gives the possibility of labelling in two different positions is shown in Scheme 4.[28] These reaction sequences enable the synthesis of ^{11}C-labelled DOPA, tryptophan, 5-hydroxytryptophan, tyrosine and some analogues thereof.

These syntheses, carried out using free or immobilised enzymes, start with ^{11}C-labelled DL-alanine, obtained through conventional synthesis. The alanine, labelled in the carboxylic or the methyl position, is then transformed to the aromatic amino acid within 3–5 min, giving radiochemical yields sufficient for PET investigations. The conversion of the labelled DL-alanine to pyruvate is an example of a selective oxidation by D-amino acid oxidase (DAO) and glutamic puruvic transaminase (GPT). The following C–C bond-forming reactions between the aromatic precursor and pyruvate are performed by β-tyrosinase and tryptophanase, respectively. The intermediate obtained, pyruvate labelled either in the carboxylic or the methyl position, is also a starting material



Scheme 4.



Scheme 5.

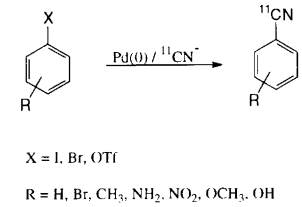
for the enzymatic reduction to L-lactate[29] as well as an interesting tracer itself.

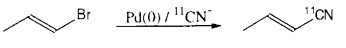
All the reactions described above are stereoselective and give the products in enantiomeric purities higher than 99%. Other examples of C–C bond-forming, enzyme-catalysed reactions are shown in Scheme 5. In this case a substitution reaction between [^{11}C]cyanide and *O*-acetylserine and *O*-acetylhomoserine results in two labelled nitriles.[30] These are further transformed, enzymatically or by conventional synthesis, to the corresponding primary amines, amides or carboxylic acids. Through this route it is thus possible to ^{11}C-label the following amino acids: aspartate, asparagine, 2,4-diaminobutyric acid, glutamine, glutamate and ornithine.

The enzyme-catalysed substitution reaction is stereoselective with respect to the selectivity of the L-enantiomer of the precursor. So far only aspartate and glutamate have been used in PET investigations. Enzymes are particularly useful in labelling synthesis where other methods either fail due to the sensitivity of the compounds or because the time factor prevents the use of ordinary chemical methods.

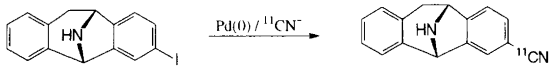
Palladium-mediated cyanation reactions using hydrogen [^{11}C]cyanide

Palladium-mediated coupling reactions have allowed the formation of many kinds of carbon–carbon bonds that were previously difficult to make. One example is the synthesis of ^{11}C-labelled aromatic[31] and vinylic[32] nitriles via the palladium-mediated reaction of [^{11}C]cyanide and an aromatic or vinylic halide (Scheme 6). Nitriles are valuable synthetic intermediates due to the ease of further transformation into other functional groups such as carboxylic acids, amides or amines. One application is the ^{11}C-labelling of an analogue of the NMDA-receptor antagonist MK-801[33] (Scheme 7).





Scheme 6.

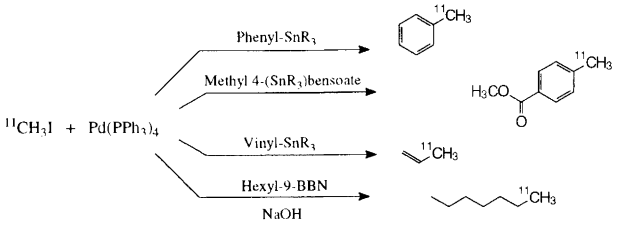


Scheme 7.

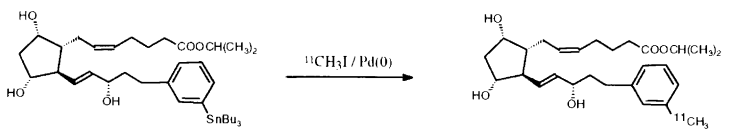
Palladium-mediated cross-couplings using [^{11}C]methyl iodide. Palladium-mediated cross-coupling reactions are important tools in organic synthesis. Recently this methodology was investigated in the synthesis of ^{11}C-labelled compounds, via the Stille and the Suzuki reactions[34] (Scheme 8). The Stille reaction is the cross-coupling of an organotin reagent with an organohalide and the related Suzuki reaction is the coupling of an organoboron compound with an organohalide. Since most functional groups are tolerated, and thus protective groups can be avoided, these reactions are of particular interest for synthesis using short-lived radionuclides.

The Stille reaction has proved to be a reliable method for the ^{11}C-labelling of a wide array of different substances including several different prostaglandin and prostacyclin analogues[35] (Scheme 9). In some cases, however, when sensitive and high molecular weight compounds are involved, the preparation of the tin reagent may present problems. For this reason, a synthesis of an ^{11}C-labelled tin compound was developed as an alternative method. It has been shown that a methyl group bound to 1-aza-5-stanna-5-methyltricyclo[3.3.3.0]undecane is easier to transfer in cross-coupling reactions, compared to a methyl groups on an ordinary tetramethyltin compound.[36] The new ^{11}C-precursor, 1-aza-5-stanna-5-[^{11}C]methyltricyclo[3.3.3.0]-undecane, was prepared from [^{11}C]methyllithium and used in a model reaction (Scheme 10).

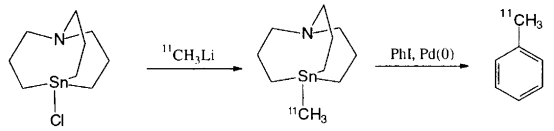
Palladium-mediated vinylic substitution of ^{11}C-labelled alkenes. The palladium-mediated cross-coupling of vinyl or aryl halides with alkenes (the Heck reaction) has been shown to be a particularly versatile reaction for carbon–carbon bond formation. Since ^{11}C-labelled alkenes can be synthesised in high yields, using the Wittig methodology described previously, the Heck reaction opens some interesting possibilities. The approach permits the incor-



Scheme 8.



Scheme 9.

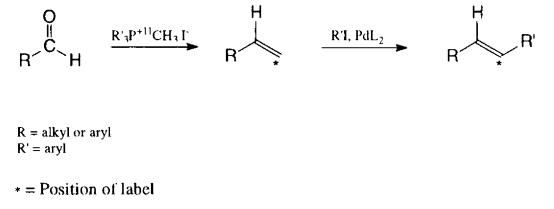


Scheme 10.

poration of ^{11}C in complicated carbon structures inaccessible by established labelling methods (Scheme 11).

The subsequent Heck reaction can be performed without purification when tri(*o*-tolyl)phosphine is used in the Wittig reaction, instead of triphenylphosphine. The reason why triphenylphosphine, obstructs the reaction, is that it causes ligand exchange to take place with the palladium after the oxidative addition.[37] The result of the ligand exchange is a coupling product with a phenyl group from the triphenylphosphine, rather than the group from the organohalide. The Heck reaction has been used in the synthesis of several ^{11}C-labelled olefins with aromatic substituents (Scheme 12).

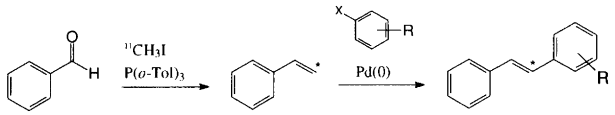
Organocuprates. Coupling reactions with organocuprates were studied with the prime objective to develop methods for ^{11}C-labelling of fatty acids in selected positions. Bis-Grignard reagents were used in coupling reactions with ^{11}C-labelled alkyl iodides in the syntheses of a broad range of saturated fatty acids (Scheme 13).[24a,c] It was also possible to label the polyunsaturated fatty acid, arachidonic acid.[24b] This method was, however, not



R = alkyl or aryl
R' = aryl

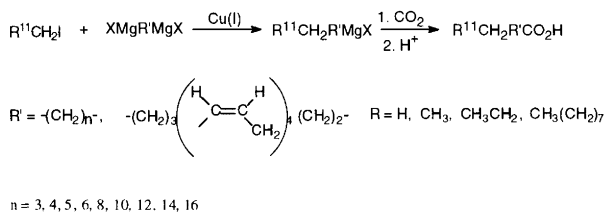
* = Position of label

Scheme 11.



R = NH_2, CH_3, $COOCH_2CH_3$, CH_2OH
X = I, Br
* = Position of label

Scheme 12.



n = 3, 4, 5, 6, 8, 10, 12, 14, 16

Scheme 13.

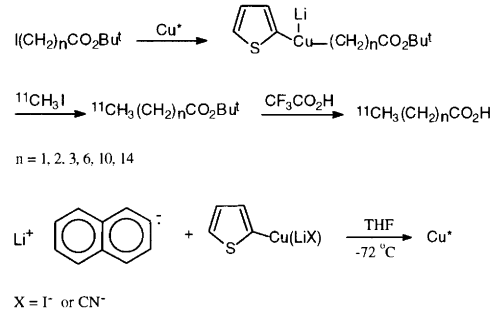
suitable for the labelling of other unsaturated fatty acids, e.g. linoleic acid.

The idea of obtaining organocuprates *in situ*, directly from the corresponding organohalide, were investigated in order to develop a more general method for ^{11}C-labelling of saturated and unsaturated fatty acids. It was found that zero-valent copper reagents (Cu*), obtained from thienyl cuprates, were suitable.[38] This approach permits substrates containing a range of functional groups that are incompatible with Grignard or lithium reagents (e.g. carbonyl compounds and nitriles).[39] Several saturated fatty acids as well as linoleic acid were labelled with ^{11}C in the methyl group using this method (Scheme 14).[24d,e]

^{11}C-Labelled methyl-2-thienyl cuprates were obtained from [^{11}C]methyl iodide, via conversion to [^{11}C]methyllithium and a subsequent reaction with lithium 2-thienyl cuprates (A) or by a one-step reaction using the zerovalent copper complex described previously (B) (Scheme 15). The lithium [^{11}C]methyl(2-thienyl)cuprates were studied in the syntheses of ^{11}C-labelled octane and acetophenone[10] and applied in the syntheses of [21-^{11}C]progesterone[40] and [1α-methyl-^{11}C]mesterolone[41] (Scheme 16).

^{11}C-labelling reactions using [^{11}C]carbon monoxide. Carbon monoxide may be used to synthesise nearly all types of carbonyl compounds.[42] Since carbonyl groups are common among biologically active substances the potential of [^{11}C]carbon monoxide, as a precursor in tracer synthesis, is obvious. This is further accentuated by the fact that many carbonyl compounds are versatile substrates for further chemical transformations.

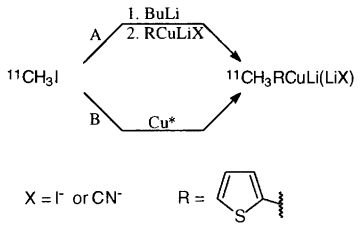
The reactivity and solubility of carbon monoxide are low in comparison with other ^{11}C-labelled precursors. For these reasons it is usually difficult to trap [^{11}C]carbon



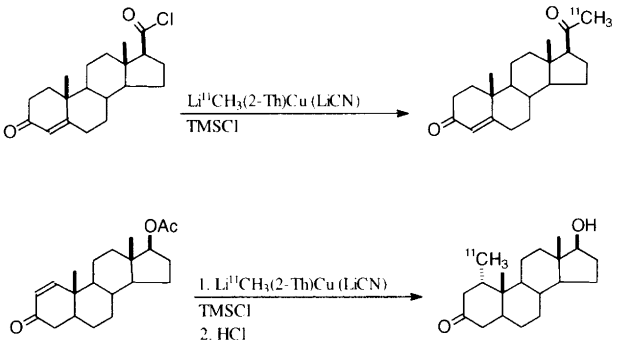
n = 1, 2, 3, 6, 10, 14

X = I^- or CN^-

Scheme 14.



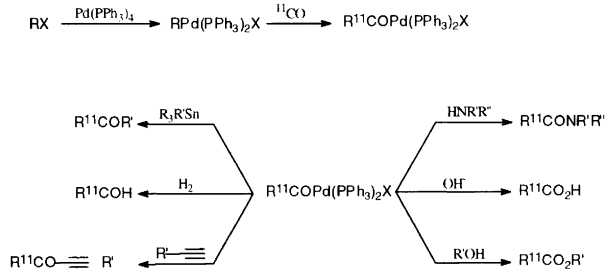
Scheme 15.



Scheme 16.

monoxide in a reaction medium.[43] Two approaches have been developed to overcome the trapping problem: (1) recirculation of [^{11}C]carbon monoxide through the reaction media; (2) concentration and enclosure of [^{11}C]carbon monoxide into a micro autoclave at high pressure. Method 1 was first studied and used in the synthesis of ^{11}C-labelled ketones.[44] Using the second approach, the scope of [^{11}C]carbon monoxide as a labelling precursor was significantly expanded. Increased trapping efficiencies, from less than a few percent to nearly quantitative values, were reached in several cases when changing from method 1 to method 2. Compounds where the carbonyl group was bound to one or two carbon atoms were prepared using palladium mediated synthesis as presented in Scheme 17. Protective groups were usually not required and the whole synthesis could be performed with a one-pot procedure. Synthesis times could then be kept in the range of a few minutes.

Some examples of compounds that were ^{11}C-labelled with palladium mediated carbonylations are shown in Fig. 2.[45]



Scheme 17.

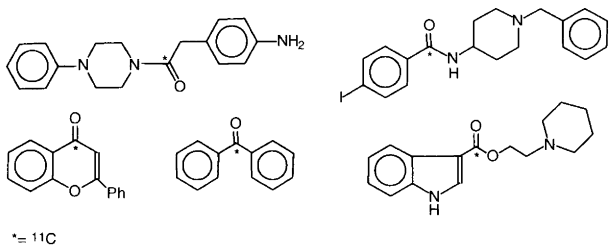


Fig. 2. Compounds labelled with ^{11}C in the carbonyl position, using ^{11}CO in a palladium-mediated insertion reaction.

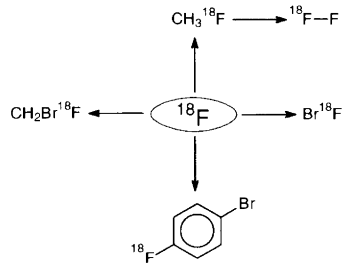


Fig. 3. ^{18}F-Labelled precursors.

Compounds where the carbonyl group was bound to two hetero atoms (e.g. ureas, carbamates and carbonates) were ^{11}C-labelled using [^{11}C]carbon monoxide in selenium mediated reactions (Scheme 18).[46] It was found that small amounts of selenium could be dissolved when heated in a DMSO solution with the substrate (amine/alcohol). The resulting solution could then be used in the carbonylation reaction. In some cases yields were markedly increased if imidazole was added. It is presumed in these cases that the reaction proceeds via [^{11}C]carbonyl diimidazole. Further work on the scope and limitation of using [^{11}C]carbon monoxide in labelling synthesis is currently in progress.

Synthesis of compounds labelled with β^+-emitting halogens. Labelling with halogens is best suited to compounds with a naturally occurring halogen, but it can also be used to make halogen analogues. In the latter case, the time frame for the study may determine the choice of halogen radio nuclide. Several useful methods for introducing the halogen have been developed, including halodemetallation, nucleophilic substitution and electrophilic substitution.

The halogens ^{18}F and ^{76}Br. Owing to its convenient half-life (110 min) and well established methods for production and incorporation, ^{18}F is by far the most used β^+-emitting radiohalogen. Relatively long reaction times are allowed, and biological processes can be studied for several hours. [^{18}F]F$^-$ is produced in the target and can be used in nucleophilic substitution reactions directly after removal of target water (Fig. 3).

Recently a method for accelerator production of ^{76}Br ($t_{1/2}$ 16 h) with low-energy protons has been developed[47] allowing studies of slow biological processes like cell proliferation. The ^{76}Br$^-$ may be used directly in nucleophilic substitution or in electrophilic substitution reactions in the presence of an oxidant.

Scheme 18.

Nucleophilic fluorination. Nucleophilic displacement of halides or other good leaving groups such as sulfonates and triflates is the most common way of introducing $^{18}F^-$. An example is the synthesis of 2-deoxy-2-[^{18}F]fluoro-D-glucose, a D-glucose analogue, obtained from 1,3,4,5 tetraacetyl-D-mannose triflate. This is the most used and one of the most useful tracers available at present.

Bromo[^{18}F]fluorination can be used to prepare compounds with ^{18}F at secondary or tertiary positions, where the nucleophilic displacement might be hindered, e.g. the labelling of 1,4-diisopropylcyclohexene and 1-methylcyclohexene.[48]

Aromatic nucleophilic substitution. A good leaving group, e.g. nitro, iodo, bromo or triflate, and at least one electron withdrawing group needs to be present in order to perform aromatic nucleophilic fluorination. In the synthesis of 4-bromo-[^{18}F]fluorobenzene the aldehyde group activates the substitution reaction (Scheme 19).[49] The labelled aldehyde is then decarbonylated to form 4-bromo-[^{18}F]fluorobenzene. This compound was used in palladium promoted cross-coupled reactions to introduce 4-[^{18}F]fluorophenyl. The 4-fluorophenyl group is found in many pharmaceuticals.

Electrophilic halogenation. Both F^- and Br^- can be transformed into their electrophilic counterparts. In low specific activities, F_2 is obtained from F^- and methyl iodide via MeF reacted with F_2/Ne in a high-voltage discharge chamber. With a suitable oxidant such as chloramine-T Br^- is oxidized to BrCl.

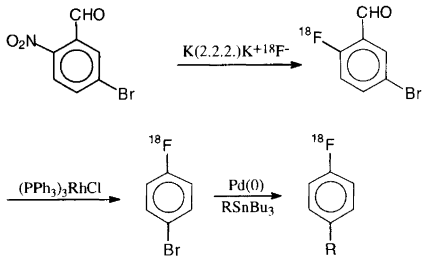
Halodemetallation with vinylic or aromatic tin compounds is a fast, high yielding and regioselective reaction for introducing ^{76}Br or ^{18}F.[50] An example, the synthesis of 5′-[^{76}Br]bromodeoxyuridine, is shown in Scheme 20.

Nucleophilic substitution reactions and electrophilic bromination on aromatic compounds are alternative methods for introducing bromine.[51]

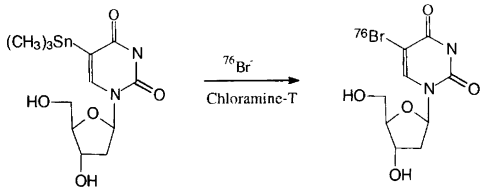
Conjugation labelling of macromolecules. Oligonucleotides, proteins and peptides are important molecules in life sciences and are therefore interesting in PET contexts. Proteins and peptides are directly labelled by bromine on the tyrosine residue by oxidative methods. For oligonucleotides and proteins lacking tyrosine residues or that are sensitive to oxidative conditions, conjugation to a small labelled prostethic group is an option available. Conjugation is a mild labelling technique that can be performed in aqueous solutions. The same prostethic group can be used for a variety of substrates and for different radiohalogens.[52] Macromolecules have been labelled with [^{76}Br]bromide via conjugation using *N*-succinimidyl 4-trimethyl-stannylbenzoate (Scheme 21).[53]

A similar procedure has been used to incorporate ^{18}F in oligonucleotides.[54] In this procedure ^{18}F was introduced in the prostethic group by nucleophilic substitution with $^{18}F[F^-]$. The reaction was performed on an activated aromatic ring which was further converted to a succinimidyl ester (Scheme 22).[55]

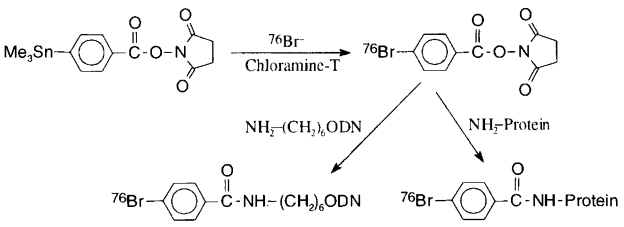
Groups other than succinimidyl esters, that can bind to amines, have also been investigated. Fluorination by nucleophilic substitution and then coupling of the isothiocyanate to the oligonucleotide has been explored (Scheme 23).[56] This fluorination is more straightforward compared to the synthesis of *N*-succinimidyl 4-[^{18}F]fluorobenzoate, but suffers from defluorination due to solvolysis in the conjugation step.



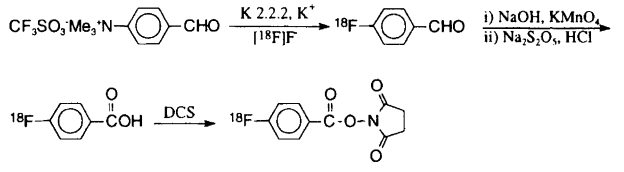
Scheme 19.



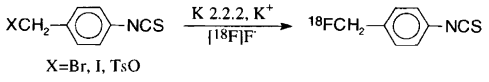
Scheme 20.



Scheme 21.



Scheme 22.



Scheme 23.

Positron emitting tracers applied as a tool in science

Applications in analytical science. The short-lived positron emitting radionuclides have been applied not only in the life sciences but also in disciplines such as physical organic chemistry and analytic chemistry. In a separate article in this issue the use of ^{11}C and ^{18}F in reaction mechanism studies is described.[57] In the remaining part of the article we will briefly bring up the advancement of positron emission tomography as a diagnostic tool in nuclear medicine. This has been covered extensively elsewhere,[4] and we will only discuss some aspects of PET and its application in drug development and in analytical science. Examples from preclinical, phase I and II clinical trials in drug development will be presented. An important theme in these applications is the utilisation of the dualistic decay properties of the positron emitting radionuclides (i.e. the β- and the γ-radiation).

Radiodetection as a tool in column technology. Chromatographic peak shape is generally seen as a measure of column performance and sometimes as a diagnostic tool. It not only illustrates column performance but also is representative of peak broadening during injection and detection and other irregularities or misfunctions in the chromatographic system.

Using short-lived radio tracers with chromatography gives the opportunity to study on-column events and interactions such as band broadening and sample loss. This technique provides the means to study the processes occurring throughout the system rather than the sum of processes as seen via conventional detection. The absorption of a small fraction of the analytes by irreversible or slow reversible adsorption on the column material can be observed as the analyte band proceeds through the column (Fig. 4).[58] This is clearly shown by an increased background signal on the developed image from the part

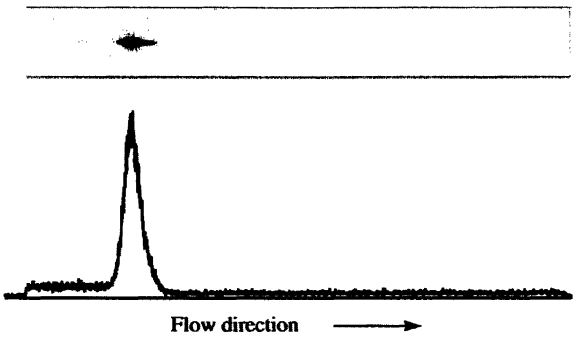


Fig. 4. An image of the distribution of [^{11}C]methyl iodide in a reversed phase packed capillary column at a specific time during elution. It can be seen that the background has increased in the part of the column the sample has passed through, indicating that a small amount of the sample is adsorbed in the column.

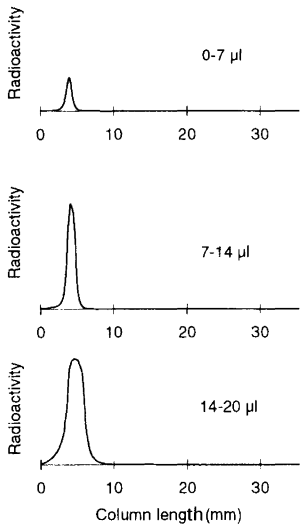


Fig. 5. 'On-column focusing' monitored by imaging of the radioactivity during a 20 µl injection onto a column with 0.2 mm I.D. The injected solution consisted of 15 µM *N*-[^{11}C]methyl-4-piperidyl benzilate. The radioactivity was monitored during three different time periods to determine analyte distribution changes with injected volume.

of the column through which the analytes have passed. In addition, it is possible to study the band-broadening occurring as the sample components advance through the column.

The use of tracers labelled with short-lived radionuclides in analytical chemistry may generate valuable novel information about the physical and chemical processes that are limiting the analytical technique. Images of the distribution of radioactivity can be made at any given time since the system under study will not be contaminated with radioactivity more than a couple of hours. Scanning the radioactivity in the column can be used to observe the distribution of the substances lost in column, connections and column end frits.

The process of 'on-column focusing' has also been studied using radioactive tracers (Fig. 5). The focusing technique is frequently used for injection of dilute samples in order to concentrate the sample prior to detection. Analytes injected in a non-eluting solvent are retained at the beginning of the separation column and elute when a stronger solvent is passing through. The extent of pre-concentration is difficult to estimate, and will depend on the volume and quantity of the analyte as well as the sample matrix. From injection of different

volumes an estimate of the maximum possible injection volume may be interpolated. Similar radiotracer experiments can be used to model solid-phase and supercritical fluid extraction methods. Measurements of recovery and loss are facilitated in such cases.[18e]

PET as a tracer method in drug development

The development of a new drug is time-consuming and expensive. The costs involved increase markedly the closer the process proceeds to market approval. This is especially noticeable in the phases of clinical trials which occupy a significant part of time and cost. During drug development there are a number of crucial decision points. It is necessary to consider routes for further promotion of the project, and in the worst case to consider its termination. At each of these points, adequate information must be available to give the optimal base for decision. The relevance of preclinical studies (e.g. in genetically modified cells or small animals) to drug behaviour in man is a key issue in the first phase I and phase II clinical trials. PET in combination with *in vitro* studies can bridge the gap between the specific biology in cell and tissue and the complicated, integrated biology in man. For these reasons, PET is increasingly applied in different areas of drug development.

From in vitro *to* in vivo. There has been a significant development of *in vitro* methods for the characterisation

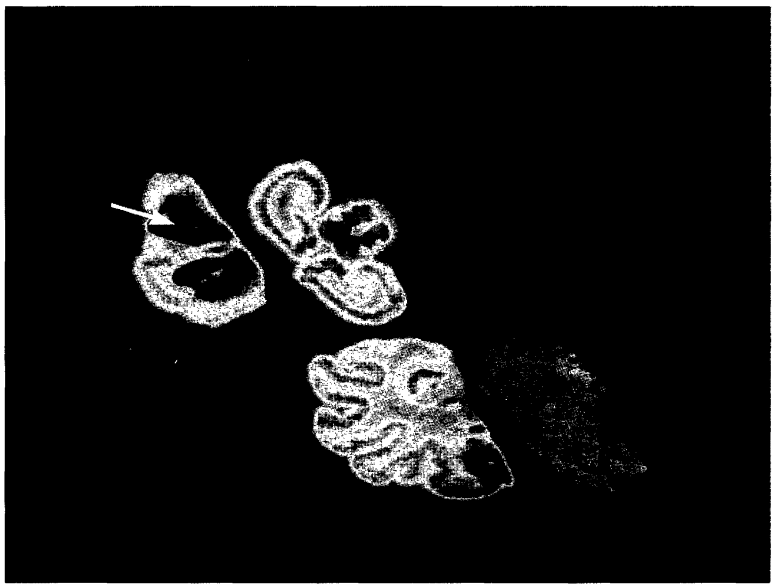


Fig. 6. Frozen section autoradiography obtained through incubation of 30 μm thick sections of brains from marmoset monkey and pig, with a ^{11}C-labelled ligand, specific for the NK1-receptor system. After incubation for 30 min, the sections were washed three times, dried and exposed for 40 min on a phosphorus imaging plate. The image shows the highest specific binding in striatum followed by cortex. Binding to cerebellum is very low. This study is part of a preclinical characterisation of the NK1-ligand and also used as a comparison of *in vitro* and *in vivo* binding pattern.

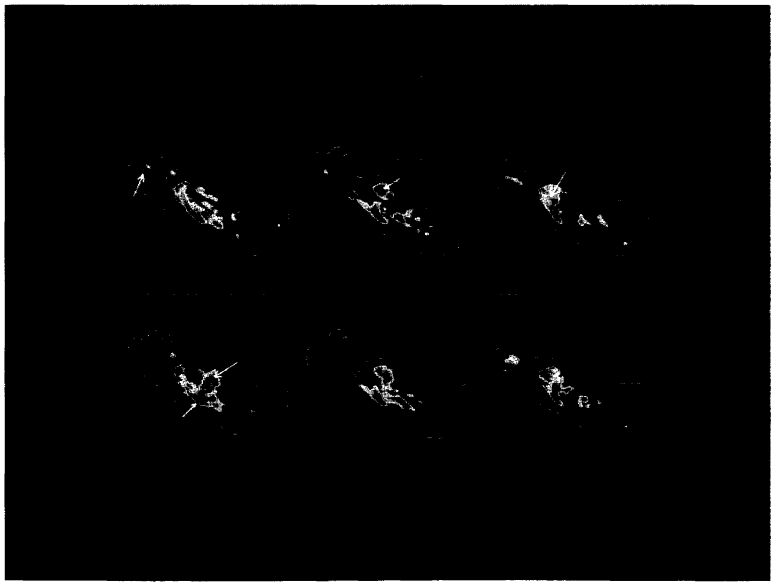


Fig. 7. In vivo distribution of a ^{11}C-labelled NK_1-antagonist in sagittal sections of Rhesus monkey. This study is a kinetic study made at five different doses (two of them shown) from tracer level with a few micrograms injected, up to assumed therapeutic doses with 0.5 mg/kg administered. A non-linear organ kinetics is illustrated by a higher amount of radioactivity in liver and lower in lung at high doses.

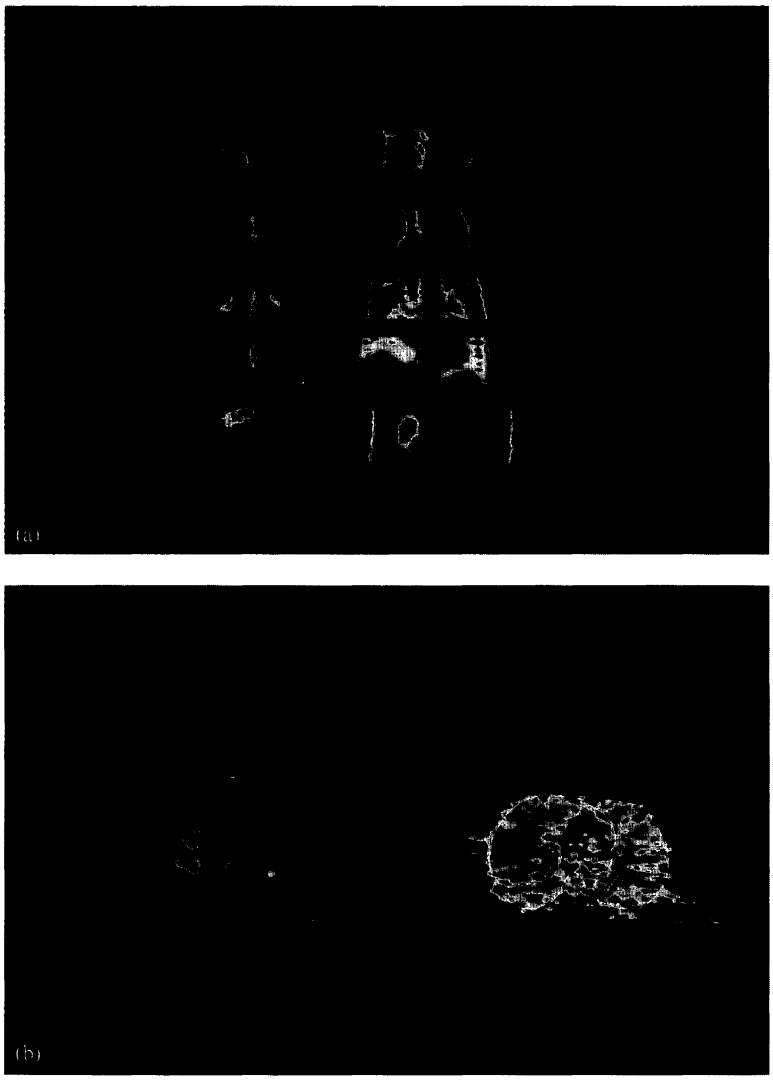


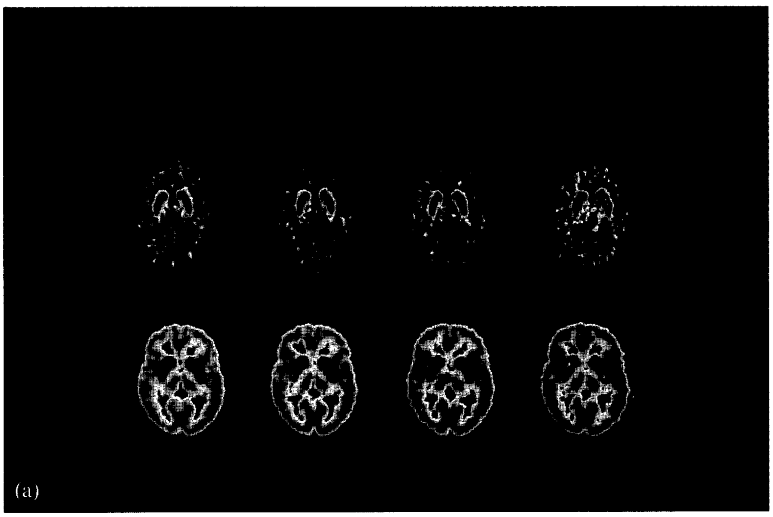
Fig. 8. (a) Deposition of [^{11}C]nicotine from a nicotine inhaler to be used for smoking cessation. (b) Lack of lung deposition from the inhaler, as compared to [^{11}C]nicotine from a cigarette, supports the kinetic data suggesting oral deposition followed by slow absorption in the gastro-intestinal tract.

of PET tracers. Prior to use in humans, tracers are defined with respect to its receptor and enzyme binding properties, kinetic behaviour, specificity and competition with other compounds, under the simplified conditions which exist *in vitro* or *in vivo* in small animals (Fig. 6). This type of information is essential for an assessment of compounds likely to be applied *in vivo* in humans. The information is also important for adequate interpretation of the *in vivo* results. These explorations might furthermore promote ideas about new applications of a given PET tracer. Sometimes the drugs are labelled with a β^+-emitting radionuclide, and in other cases the labelled compounds have a specificity for a given biochemical system to monitor the drug effect.[59]

Some of these studies might be performed with substances labelled with conventional radionuclides such as ^{3}H or ^{14}C. In many cases, however, PET tracers are advantageous. PET radionuclides have specific advantages for certain applications (e.g. high specific radioactivity). More important, the experiences gained *in vitro* may readily be extended to *in vivo* studies in primates or in humans, once a labelling method has been established.

Drug distribution. Knowledge of the distribution of a drug in the body is essential for assessment of the possible desired or non-desired interactions in different tissues. This type of information is not readily attained in humans, and except for PET studies, it has to be derived indirectly from mathematical models with input from plasma pharmacokinetics and extrapolation from animal data. These extrapolations are often uncertain because distribution, plasma protein binding, metabolism in different organs and binding to receptors and enzymes are often markedly different between species as well as between different individuals of the same species. Significant changes in all the factors modulating drug distribution may also occur in disease or after pharmacological treatment with other drugs.

PET gives the possibility to measure the tissue radioactivity and correlate that to drug concentration and



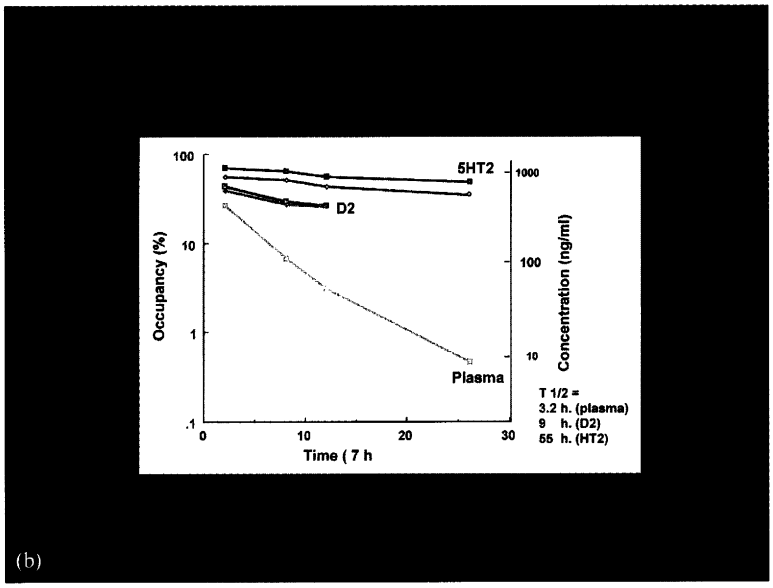


Fig. 9. Evaluation of degree of inhibition of dopamine D_2-receptors using [^{11}C]raclopride and serotonin HT_2-receptors using [^{11}C]*N*-methylspiperone in patients with schizophrenia. The receptor occupancy had a much longer duration in the brain than predicted from plasma pharmacokinetics, but was also different between the two receptor systems. (a) PET images showing receptor occupancy in the brain at different times after last dose of the drug. (b) Comparison of the plasma pharmacokinetics of the drug and occupancy of brain dopamine D_2 and serotonin HT_2 receptors.

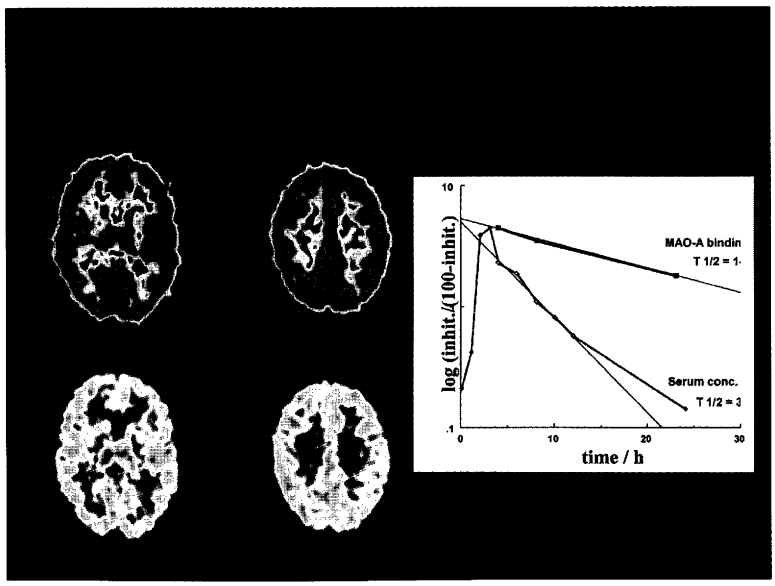


Fig. 10. Evaluation of inhibition of MAO-A by a new antidepressive drug. The PET tracer [^{11}C]harmine was developed as an indicator of the amount of free enzyme in the brain. In a double blind placebo controlled clinical trial, it was shown that esuprone at suggested dose inhibited brain MAO-A by 80%, similarly to a drug on the market. Pharmacokinetics suggested a half-life in serum of 3.5 h whereas the effect in the brain remained with a half-life of 15 h.

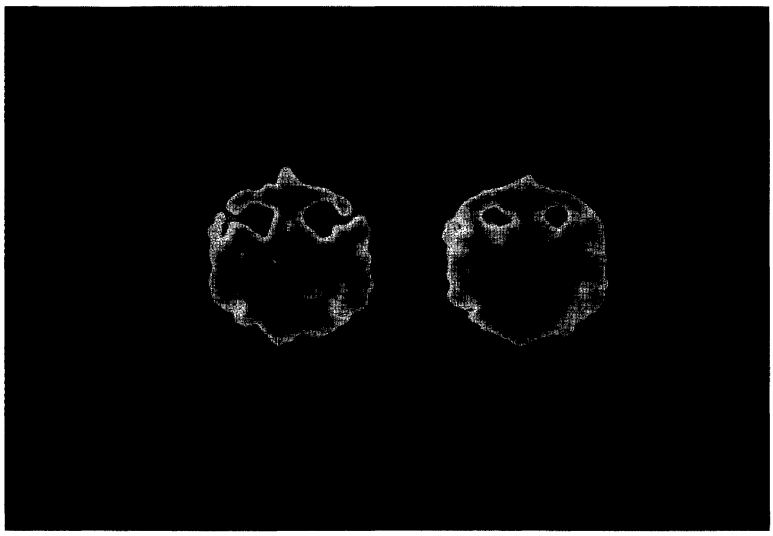


Fig. 11. Dopamine synthesis in monkey brain as evaluated with L-[^{11}C]DOPA labelled in the β-position, leading to formation of [^{11}C]dopamine which is stored in secretory vesicles, and labelled in the carboxy position, leading to loss of radioactivity as $^{11}CO_2$. The position specific labelling unequivocally shows the enzymatic activity of aromatic amino acid decarboxylase in the brain.

allows the observation of temporal changes with high accuracy and precision (Fig. 7). There are, however, two major limitations in the use of PET for evaluation of drug distribution. The tissue kinetics can only be monitored during 4–6 $t_{1/2}$ of the radionuclide. This implies that substances labelled with ^{11}C may be monitored maximally up to 1.5–2 h, with ^{18}F 8–12 h and with ^{76}Br 2–4 days. Although the radioactivity has decayed by a factor of 16–64 at the end of such an investigation, the signal is often still large enough for a quantitative determination of tissue radioactivity concentration, even if the image quality is markedly impaired. Another major limitation is that PET is measuring the total radioactivity concentration but cannot discriminate different chemical species. It is therefore not possible to decide if uptake in a tissue is dominated by the native drug or by its metabolites. Some guidance may be obtained from metabolite analyses of plasma, comparisons with animal experiments and in selected cases by comparisons between experiments performed with the tracer molecule labelled in alternative positions.[4]

Regional pharmacokinetics, non-linear tissue kinetics. A PET study in humans to evaluate the tissue kinetics in interior organs is performed with the injection of a tracer dose of the labelled compound. A human study can be performed with a few micrograms of the drug owing to the very high specific radioactivity (radioactivity per mass unit). The low injected amounts mean that documentation for non-toxicity is different than in normal clinical trials. It is not sure that the kinetics observed at low concentrations of an injected drug are relevant for the conditions existing at clinical doses and long-term treatment. Some drugs may have a proportionally higher or lower concentration in certain organs when the administered dose is increased (Fig. 7). The reason for this non-linearity may be saturation of a receptor system, saturation of an enzyme system (e.g. one which is metabolising the drug), pharmacological effects on an organ (e.g. with respect to perfusion), or saturation of plasma protein binding. It is therefore advisable to perform kinetic studies at a few different dose levels in the vicinity of the assumed clinical dose. As mentioned previously, PET has a very high quantification accuracy and may determine the tissue concentration with excellent precision. Usually the inter-individual variation in kinetics is significantly larger than the measurement precision.

Passage over the blood–brain barrier. A special application of pharmacokinetic studies with PET is the evaluation of passage over the blood–brain barrier. An assessment of the passage into the brain parenchyma is of course of utmost interest in the characterisation of all neuroactive drugs, or drugs which might have neurologic or psychiatric side effects. Passive diffusion over the blood–brain barrier occurs readily with lipophilic compounds. Lipophilicity is, however, not the only decisive criterion. A number of other factors will influence the degree of passage into the brain, e.g. plasma protein binding and its reversibility or peripheral metabolism. Furthermore, the ability of a drug to enter the brain is changed in different diseases. PET is an excellent method for these types of studies and is performed in research animals.[60]

Inhaled substances. Studies of the deposition of inhaled compounds in humans have so far mainly been made with scintigraphy. A strength of this method is that the major parts of the respiratory system can be monitored simultaneously due to the large field of view of the gamma camera. There are a number of limitations in this method which makes PET a strong alternative. PET using the labelled active component allows its deposition and disposition to be monitored. With scintigraphy usually only the deposition of a labelled surrogate, a particle or a biologically inert compound can be used.[61] PET has a significantly higher spatial resolution, and through the simultaneous acquisition of multiple tomographic

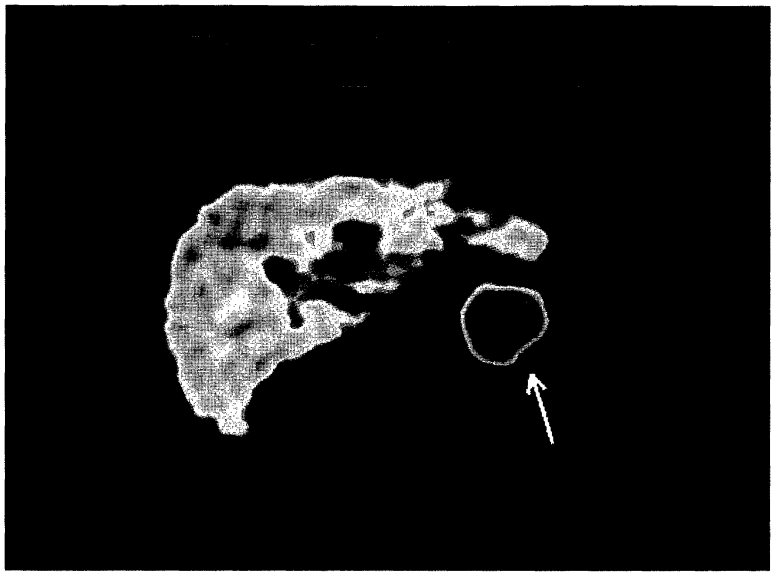


Fig. 12. Patient with accidentally found mass at the site of the adrenal, so called incidentaloma. PET was performed with [^{11}C]metomidate, a tracer specific for 11β-hydroxylase, an enzyme expressed only in the adrenal cortex and in related tumours, and shows that the mass is an adrenal adenoma and not a metastasis. The patient will be handled as harbouring a benign adrenal tumour.

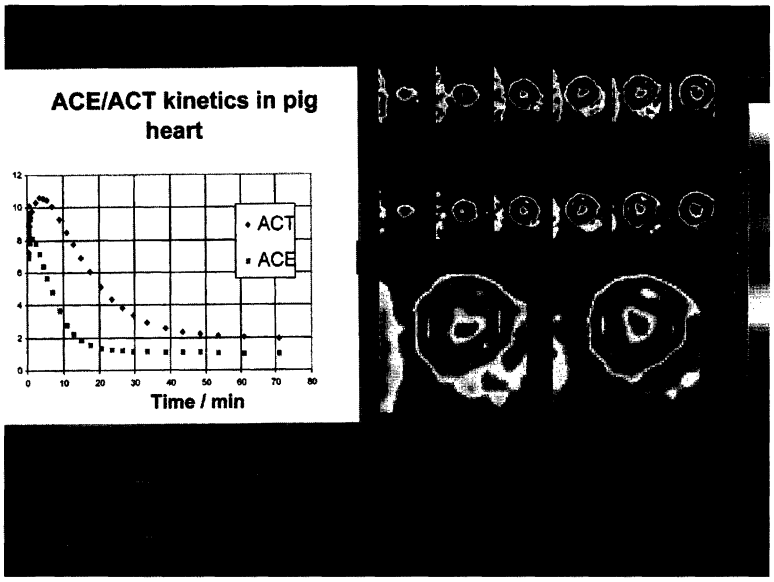


Fig. 13. Position specific labelling of acetate is used in studies of the human heart to assess TCA cycle activity *in vivo*. Labelling in carboxy position (ACE) means that the label is lost as $^{11}CO_2$ during the first round of the TCA cycle, indicated by the rapid elimination kinetics. Labelling in the 3-position (ACT) means that the label is lost in the second turn in the TCA cycle, as indicated by a slower elimination kinetics.

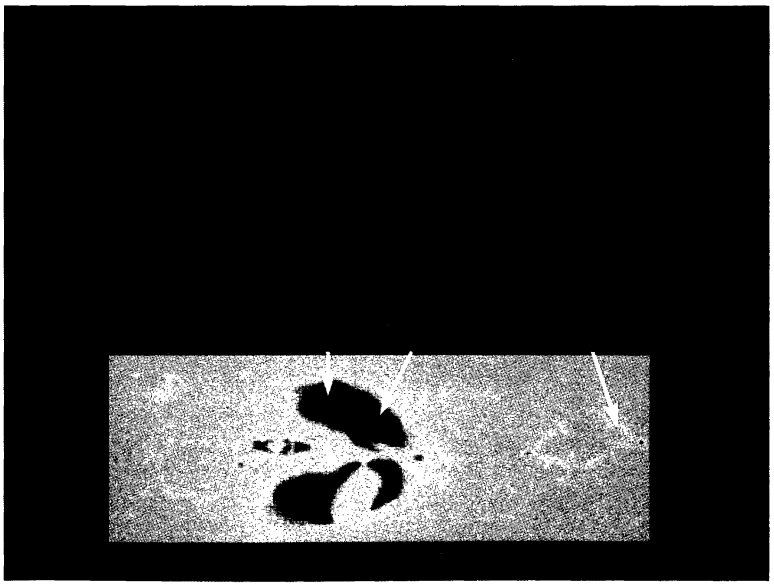


Fig. 14. Distribution of ^{76}Br-labelled antisense phosphorthioate oligonucleotides in the rat. Depending on the length of the oligonucleotide, the uptake pattern is dominated by the kidney (shorter oligonucleotides) or the liver (longer oligonucleotides). This is a preclinical demonstration of the potential of PET to assess oligonucleotide pharmacokinetics *in vivo*.

sections, a three-dimensional distribution is observed. Combined with the very high quantitation accuracy, PET allows an excellent delineation of the concentration within the lung (Fig. 8). Some compounds experience a rapid disposition, e.g. from the oral cavity to the stomach or from lung parenchyma to blood. PET can give a high temporal resolution if desired, although confined to one field of view, 10–15 cm axially. It is possible to move the field of view relatively rapidly and hence cover different sectors of the body and thereby allowing the viewing of extended parts of the body. The studies are performed with administration of very low amounts of radioactivity, 5–20 MBq, thereby reducing the radiation dose.

Uptake over the intestinal mucosa. The usual way of administering PET tracers is via i.v. injection. Most pharmaceuticals are, however, given orally, and it is therefore desirable to study body uptake after oral administration. PET studies following oral administration have so far been made to a very limited extent. Studies of this type are feasible, depending on the fact that PET has a very high sensitivity and may monitor very small fractions of radioactivity, especially if it is highly concentrated as after oral administration. A study performed to quantitatively determine the passage of drugs over an epithelial cell monolayer *in vitro*[62] has been compared to the uptake over the duodenal mucosa *in vivo* in monkey, illustrating the potential to correlate *in vitro* and *in vivo* methods.[63]

Interaction with biochemical targets. A number of PET tracers have been developed and validated allowing the *in vivo* monitoring of interaction or binding to specific receptors and enzyme systems. This gives the possibility to measure the amount of free receptors in a certain anatomical structure at one occasion, and evaluate changes induced in this receptor population during drug treatment. For drugs where a defined biochemical target is assumed, this gives possibilities to verify that the target system is indeed affected and to assess the degree of interaction. Other biochemical systems than the primary target might also be monitored for assessment of potential side effects.[64]

Dosing based on receptor occupancy. The use of PET for the evaluation of degree and duration of receptor occupancy in relation to dose has become standard for new antipsychotic and antidepressive drugs (Fig. 9). Important information derived from such studies include the following: (i) drug interaction with the assumed biochemical target and hence information that the drug reaches the target tissue, e.g. passes through the blood–brain barrier; (ii) degree of receptor occupancy reached for a given dose, i.e. the dose–occupancy relations; (iii) duration of action on the target system and its relation to conventional plasma kinetics; (iv) variability in a patient population with respect to receptor occupancy; (v) degree of receptor occupancy affected by other drugs. Some of these factors are essential for a decision on dose and dose interval in a clinical trial.

Relation between pharmacokinetics and pharmacodynamics. Pharmacokinetic data are frequently obtained in clinical trials, with the assumption that such data can be used for the prediction of dose and for the assessment of inter-individual variability. A fundamental problem with these data is that they may not well reflect the effect on the target system, neither with respect to degree or duration following a single dose of the drug. In many cases there are significant discrepancies between the duration of action and rate of elimination of the drug from plasma (Fig. 10). This implies that in order to adequately interpret pharmacokinetic data, a model should be formulated including the relation between pharmacokinetics and pharmacodynamics. With an extended model, the pharmacokinetic data obtained in a clinical trial can be used with better confidence and the model used for predictions.

Evaluation of drug effect. PET might allow the measurement of a physiological parameter correlating with tissue function and which then can be used as a surrogate marker for assessment of treatment effects.[65] In Alzheimer's disease clinical scoring with psychometric tests is affected by a large variability, since long follow-up times and large patient groups are needed to record statistically significant effects. In a number or clinical trials, brain function as expressed in glucose metabolism rate or cerebral blood flow has been used as objective and quantitative criteria of treatment effect as in Alzheimer's disease.[66]

In oncology metabolic markers such as ^{18}F-fluorodeoxyglucose or ^{11}C-methionine give an accurate description of anti-tumoural effect following treatment. There are data that show that metabolic effects are recorded much earlier than effects on tumour size. This type of study can be used for 'proof of concept' at an early stage in the development of a new anti-tumoural agent. Because of the costs and limited availability we do not foresee that PET will be used on a larger scale or replace clinical endpoints,[67] but may have a significant role to supply crucial information during the development of drugs.[68]

PET in clinical practice and medical research. PET should be regarded as a general tracer method for use with various types of biological applications. Its role in the recording of biochemistry and physiology *in vivo* is highlighted by the fact that some tracer compounds are used routinely in the clinic for diagnosis and differential diagnosis and as an important contributor in decision of patient treatment strategy. In oncology PET with ^{18}F-fluorodeoxyglucose (FDG) has been demonstrated to be a sensitive method to diagnose soft tissue tumours.[69] The early use of PET has proven to be cost effective in reducing unnecessary surgery when tumour spread is

already at hand, whilst giving stronger indications for focal treatment when such spread is not discovered.[70] As well as the number of other PET tracers which been introduced with general metabolic features, such as ^{11}C-methionine, ^{11}C-acetate or ^{76}Br-bromodeoxyuridine[71] analogues, others may show high specificity for a certain pathway, enzyme or receptor expressed in the tumour, such as ^{11}C-5-hydroxytryptophan,[72] ^{11}C-L-DOPA (Fig. 11) or ^{11}C-metomidate (Fig. 12).[61a,73]

In neurology, metabolic tracers are used for monitoring of the brain residual function in stroke patients and for differential diagnosis and treatment monitoring in Alzheimer's disease.[74] Specific tracers for the dopaminergic pathways are used in Parkinson's disease[75] and other movements disorders as well as in schizophrenia.[76]

Another field of clinical use of PET is in cardiology, where heart viability after ischemic insults are evaluated with metabolic tracers such as ^{11}C-acetate (Fig. 13), ^{11}C-pyruvate, ^{11}C-lactate or ^{18}F-FDG.[4]

The list of specific clinical questions in which PET may contribute is constantly growing with the increased availability of new tracers and the exploration of their potential to reveal biological function and physiology. Most of the applications of PET in medicine today, and probably also in the future, are either in medical research of a basic nature or in explorative clinical trials. A specific advantage is that it allows a bridging between pre-clinical studies and studies in humans. The activities of PET in medical research are too extensive to give even a short summary. Instead, three examples, related to some interesting chemistry, will be given as illustrations. Via position-specific labelling of L-DOPA, enzymatic activity *in vivo* (i.e. aromatic amino acid decarboxylase) is assessed (Fig. 11). Specific position labelling also allows exploration of the TCA cycle *in vivo* in the human. Anti-sense oligonucleotides can be labelled with ^{76}Br and their distribution in the body recorded. With examples like the last it can be seen that PET is in the front line of medical and biological research. Although molecular biology has recently seen an explosive expansion, many of the present methods applied are static and do not give the necessary broad view on the complex dynamic multiple interacting systems *in vivo* (Fig. 14). PET may open keyholes through which some of this complexity can be observed in the complete and the integrated biological system including investigations in man.

Conclusion

With this presentation, which has focused on recent developments in rapid labelling chemistry at Uppsala University, we would like to salute Professor Göran Bergson for his support in the foundation of this project. We hope that we have been able to transfer a glimpse of the potential use of short-lived β^+-emitting tracers, including applications in medicine, life sciences and chemistry. As this field of science is in an early phase of its development, there is a strong need for continuing investigations on the scope and limitation of new synthetic methods, and their integration with applications in biological systems.

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