PET Radiopharmaceuticals: State-of-the-Art and Future Prospects

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In this review we provide a conceptual overview of radiopharmaceuticals containing positron-emitting isotopes, not a catalog of radiopharmaceuticals or details of syntheses. We hope to provide an integrated framework for understanding the radiopharmaceuticals that are available at this time, describing both their strengths and weaknesses, and to look forward to some of the improvements that might be anticipated in the next decade. The range of biology that can be studied with positron emission tomography (PET) radiopharmaceuticals has greatly expanded, involving more sophisticated tracers and more sophisticated data analysis. PET measurements now encompass increasingly more specific aspects of human biochemistry and physiology as described in this review. As the biology being studied becomes more complex, the demands on the radiopharmaceutical and the methods of data analysis also become more complex. New synthetic chemistry and data analysis must develop in tandem. Radiopharmaceuticals must be designed to ensure that the rate determining step that is of interest is the one reflected in the data from the radiopharmaceutical. The challenge to the PET community of chemists, biologists, and physicians is to apply new knowledge of human biochemistry for developing and validating useful PET radiopharmaceuticals that will, in turn, produce useful nuclear medicine procedures. Initially the synthesis of a compound containing a short-lived radionuclide was a triumph in itself. However as the science advances the radiochemical synthesis becomes just the first step in a long trail that terminates in the compound being used to provide data on biological processes via a well-designed PET experiment. The resulting list of compounds and experiments should be as diverse as all of human biology and pathophysiology.

Radiopharmaceuticals serve two complementary roles. The first is a pragmatic one in which the labeled compound is administered to the patient and some aberrant physiological or biochemical process leads to an abnormal distribution of the compound. When that distribution is imaged at a particular time, an experienced observer can make a medical decision based on the deviations of the image from the normal pattern. In the second role the radiopharmaceutical is a tracer for a particular physiological or biochemical process and the time course of its distribution is used to quantitate the biological process. The first role is typical of classical radiologic contrast agents and image quality is the all important criterion in evaluating the procedure. As long as the radiopharmaceutical behaves predictably *in vivo*, then the higher the spatial resolution the easier it is for the observer to detect smaller departures from the range of normal. In the second role the fidelity of the radiopharmaceutical as a tracer for the process under investigation is paramount, with the image quality a secondary concern. A visually inferior image may contain more information than a beautifully high-contrast anatomic image that is a delight to the instrument builder.

An example of this is an [^{15}O]-water image of the heart that looks much less satisfactory than one from [^{13}N]-ammonia but the information content regarding blood flow is much higher in the water image.[1,2] The dichotomy is perhaps epitomized by [^{18}F]-2-deoxy-2-fluoro-D-glucose, more commonly known as FDG, the tracer most widely used in PET and the radiopharmaceutical that is almost synonymous with positron emission tomography (PET). FDG was introduced two decades ago and provides beautiful images of the heart, brain, and tumors, all of which are a function of glucose metabolism in the organ of interest. It has clearly achieved the pivotal role in PET imaging. And yet 20 years later we are still struggling to define the relationship between FDG uptake in tissues and the glucose consumption of that tissue and what it means in terms of energy balance of both normal and disease states.[3]

The technology to make radionuclides and the chemistry to make PET radiopharmaceuticals have made significant progress over the last decade. We have progressed from the accomplishment of simply making the compounds to making them reliably on a daily basis on demand of the user. A 10-year-old review of the synthesis of [^{18}F]-FDG included more than 150 literature references[4] and yet the

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1997 Workshop on Targetry and Target Chemistry and the XIIth International Symposium on Radiopharmaceutical Chemistry both had several papers on improving reliability and yield for the routine production of the radionuclide and this important radiopharmaceutical.

Nitrogen-13 and oxygen-15, the two shorter lived radionuclides of the classical quartet of carbon, nitrogen, oxygen, and fluorine, still have a limited chemistry. But the number of compounds labeled with the other two radionuclides, carbon-11 and fluorine-18, continues to expand enormously. Several hundred compounds containing these radionuclides either have been examined or are under development as suitable tracers for a host of different biochemical or physiological processes. Additional radionuclides are being examined as suitable sources of positrons, particularly $^{60\text{-}62,64}$Cu, ^{76}Br, ^{86}Y, ^{89}Zr and ^{122,124}I.

Radiochemical labeling reactions have become much more sophisticated; [^{18}F]-fluorine gas has largely been replaced with [^{18}F]-fluoride as the fluorine precursor. Increasingly demanding reactions are being performed with high specific activity fluoride ion, including the synthesis of electron poor and electron rich aromatic compounds and some progress has been made with incorporating trifluoromethyl groups. The specific activity of the final products has become much higher as the target and chemical processes that lead to the introduction of the nonradioactive isotopes is better understood and the measurement of high specific activity products becomes more routine.

The use of labeled synthons, small molecules containing the radionuclide and prepared after the target for subsequent reaction with other compounds, has expanded the range of labeled compounds that can be prepared. The archetypal compound of this type is [^{11}C]-methyl iodide,[5] the synthesis of which was first described 20 years ago and a new gas phase synthesis, described last year, has expanded the convenience, reliability, and specific activity of this compound.[6] Carbon-11 methyl iodide is probably used more than all synthons combined, but others have been developed and their uses actively explored. These include carbon-11 labeled cyanide, urea, alkyl iodides, methyl triflate, phosgene, formaldehyde, acetyl chloride, acetone, benzyl bromide, and fluorine-18 labeled fluoroethyltosylate, fluoroacetone, fluorobenzaldehyde, fluorobenzylbromide, epifluorohydrin and fluoroethylamine.

The range of biological processes that are being studied with PET radiopharmaceuticals has greatly expanded. The first PET imaging measurements focused on blood flow using [^{15}O]-water or [^{13}N]-ammonia, and metabolism using [^{15}O]-oxygen, [^{11}C]-glucose and [^{18}F]-FDG. These biological measurements are still pursued and more sophisticated tracers and more sophisticated data analysis have been developed. PET measurements have also been extended to increasingly more specific aspects of human biochemistry and physiology as described in this review. As the biology under study becomes more complex, then the demands on the radiopharmaceutical and the methods of data analysis, the model, have to also become more accurate. There are still only two input functions from the data, tissue time-activity curves and blood time-activity curves. The radiopharmaceutical must be designed to ensure that the rate determining step that is of interest is the one reflected in the biodistribution kinetics of the radiopharmaceutical. However tightly a ligand binds to a receptor and however negligible the nonspecific binding, the data can only reflect the receptor density if the binding step is rate determining. If binding is faster than delivery then even the most beautiful image only contains delivery information; no receptor binding information is available.

In this review we will provide a conceptual overview of radiopharmaceuticals containing positron emitting isotopes. It is not our intent to provide an exhaustive catalog of radiopharmaceuticals or to provide explicit details of syntheses. Rather, we hope to provide a framework for understanding those radiopharmaceuticals that are available at this time, describing both their strengths and weaknesses, and to look forward to some of the improvements that might be anticipated in the next decade.

BRAIN RADIOPHARMACEUTICALS

The first PET radiopharmaceuticals for the brain were directed towards measuring blood flow and energy metabolism. Carbon-11 glucose, [^{18}F]-2-deoxy-2-fluoro-D-glucose and [^{15}O]-oxygen were the tracers for metabolism and [^{15}O]-water was the tracer for flow.

"Uniformly labeled" [^{11}C]-glucose was prepared photosynthetically by exposing Swiss chard leaves to [^{11}C]-carbon dioxide and then extracting

the glucose from the leaf.[7] The short exposure times required by the 20 minute half-life of carbon-11 almost guaranteed that the label was not uniformly distributed, but concentrated in the 2 and 3 positions of the glucose, the first carbon atoms liberated as CO_2 during oxidative metabolism. This resulted in the rapid release of [^{11}C]-CO_2 and, as a result, it has largely fallen out of favor, replaced by 1-[^{11}C]-glucose prepared by a chemical route.[8] The carbon in this position is not released as CO_2 until after 2½ turns of the TCA cycle.

The use of [^{18}F]-2-deoxy-2-fluoro-D-glucose (FDG)[9] is based upon the work with [^{14}C]-2-deoxyglucose by Sols and Crane who showed that 2-deoxyglucose is transported into the cell in a similar fashion to glucose and phosphorylated by hexokinase in the same fashion as glucose but the glucose-6-phosphate derivative is not a substrate for the next enzyme in the metabolic chain, 1,6-phosphoglucose isomerase.[10] The result is that the glucose-6-phosphate from FDG remains locked in the cell as the anion as long as the 6-hydroxyl remains phosphorylated. FDG was first synthesized at Brookhaven by electrophilic addition of [^{18}F]-F_2, prepared using the ^{20}Ne(d,α) ^{18}F reaction in a gas target with a small amount of carrier F_2, to triacetylglucal. The initial preparations were heroic, with great efforts expended with relatively small rewards.[11] Over the years many changes have been introduced into the synthesis. The current method of choice, developed at Julich, uses a nucleophilic displacement reaction with [^{18}F]-fluoride and provides typical yields of 50% on a reliable basis.[12] Some PET facilities now produce FDG in 1 to 2 Ci quantities on a daily basis using automated equipment operated by technicians.

FDG provides very high quality images of glucose use in the brain.[13] The relationship between the regional uptake of FDG and regional glucose metabolism is adequately described by a three compartment model derived from one developed for autoradiographic work using [^{14}C]-2-deoxyglucose by Sokoloff et al.[14,15] The model works well in normal cerebral tissue although recent work suggests that the original value of 0.5 for the *lumped constant,* the factor that corrects for the difference in affinity between glucose and FDG for the transporter and the enzyme, obtained by tissue sampling in rats, is too low for humans and a value closer to 0.8 may be more appropriate.[16] The autoradiographic work with [^{14}C]-2-deoxyglucose has been plagued by arguments about the possibility of slow dephosphorylation and the loss of label from the brain.[17] This has spilled over into the FDG literature but here it is less important as continuous sampling and kinetic modeling can actually measure k_4, the rate constant for the loss of label from the brain. With FDG the consensus seems to be that there is some loss of tracer from the tissue with time, although the mechanism for this loss is not fully understood.[18,19] Thus *in vivo* imaging techniques can provide the same data in a human brain as has been obtained from tissue sampling in animals and the ability to collect time-activity data can improve the quality of the results.

Tracers for measuring brain blood flow have been relatively non-controversial. Oxygen-15 water works very well although it may underestimate blood flow in the regions of highest perfusion.[20] The 2-minute half-life of oxygen-15 makes repeat studies in the same subject convenient and brain activation studies have been performed where multiple administrations of [^{15}O]-water are given at 10-minute intervals. Oxygen-15 water has been the work horse tracer used in the vast majority of blood flow studies in the brain. Oxygen-15 butanol synthesized by reaction of [^{15}O]-oxygen with tributyl borane[21] works better than water in the highest flow regions of the brain, but it is significantly more complex to make. Not all sites can make quantities of oxygen-15 conveniently and so [^{18}F]-fluoromethane was developed as an alternative flow tracer.[22] Fluorine-18 can also be prepared using a reactor and it can be transported to sites distant from where it was prepared. While the longer half-life (110 min) might be expected to preclude repeat studies, the residence time of fluoromethane *in vivo* is quite short, ~20 minutes, and so repeat studies are possible. The tracer has the added virtue that the volume of distribution can also be measured and corrected for when measuring blood flow and, as it is exhaled by the subject, it can be collected, reconcentrated, and used again.

One of the early applications of PET for imaging a more specific brain function used [^{18}F]-fluoro-DOPA for studying dopamine utilization.[23] Fluoro-DOPA crosses the blood brain barrier, whereas fluorodopamine does not, and it is decarboxylated by aromatic amino acid decarboxylase to fluorodopamine inside the dopaminergic neurons. [^{18}F]-Fluorodopamine then traces the release, re-uptake, and metabolism of dopamine.[24,25] [^{18}F]-Fluo-

roDOPA has been labeled at positions 2, 5, and 6, initially using electrophillic fluorinations with F_2 or acetyl hypofluorite. The reactions gave low yields of mixtures of isomers which had to be separated. The introduction of fluorodemetalation reactions has subsequently solved the isomer problem and improved the yield and [F-18]-6-fluoroDOPA is now made reliably on a routine basis in a number of laboratories.[26] The different isomers all follow the same basic pathway of neuronal decarboxylation, but undergo different degrees and positions of methylation of the catechol hydroxyl groups. These O-methylated products are formed outside the brain but then are transported into the brain were they complicate the interpretation of the data.[27]

As a complement to the [^{18}F]-fluoroDOPA studies, other aspects of dopaminergic neurotransmission can now be imaged. Neurotransmission mechanisms generally involve a precursor to the neurotransmitter entering the neuron and being converted to a biologically active form which is then stored in vesicles inside the neuron. When a signal has to be passed across the synaptic cleft the neurotransmitter is released into the synapse where it binds to a receptor on the postsynaptic neuron. This receptor binding evokes a cascade of events that includes the ion transport that sends a signal to the next neuron. The neurotransmitter molecules remaining in the synapse are then either enzymatically degraded or are salvaged on the presynaptic side of the neuron by a specific re-uptake process and stored in the vesicle.

With the dopaminergic neurotransmission system there are tracers for almost all of the processes involved. [^{18}F]-6-FluoroDOPA is a useful tracer for dopamine synthesis and [^{11}C]-tetrabenazine and related molecules image the vesicular transporter.[28,29] A host of tracers are available for the postsynaptic receptor, most commonly for the D_2 subtype, but there are some for the less abundant D_1, D_3, and D_4 receptors.[30] [^{11}C]-Cocaine and related tropanes are useful for imaging the re-uptake process[31-33] and [^{11}C]-deprenyl has been developed for imaging the activity of the degradative enzyme MOA-B.[34] A recent review of PET studies of the dopaminergic system contains more than 250 references.[30]

The first PET tracer for the postsynaptic dopamine receptor was [^{11}C]-N-methyl spiperone, prepared at Johns Hopkins.[35] This compound provides beautiful high-contrast images of the regions of the brain rich in dopamine receptors. Nonetheless, it proved hard to extract accurate receptor concentrations from these images. By contrast [^{11}C]-raclopride provided lower contrast images of dopamine-rich tissues because the tracer washes in and out of the regions of interest quite rapidly, but accurate information on receptor concentration could be extracted from the data.[36,37] These tracers are providing valuable insight into the workings of the dopamine system and its involvement in drug abuse and addiction. Nevertheless, with all this imaging information about the dopaminergic central nervous system, PET has not made the anticipated large impression on the two major diseases involving the dopamine system, schizophrenia and parkinsonism. The information available from current PET tracers has made it clear that, although the effects of the diseases are obvious, the biochemical malfunctions that cause them remain elusive. New tracers and methods will have to be developed that more completely reflect this subtlety and render it accessible to measurement. Compounds that don't work very well can still supply information as to why they don't work and this can be used to make tracers that will work better. Only when the properties of an inadequate compound are understood can new improved versions be pursued. Progress in PET imaging is probably better served by trying to understand the strengths and weaknesses of available tracers and then using this insight to prepare new and improved compounds, rather than preparing another dozen ligands that bind to some receptor of interest.

Other neurotransmitter systems, such as those of the serotonin, GABA, muscarinic and nicotinic cholinergic, cannibinoid and opiate neurons, have similar mechanistic structures but the tracers for each of the different parts of these systems are not as well developed as they are in the dopamine system. It may well be that all of these neuronal systems will have tracers developed to follow each aspect of their function, but they may be more difficult to develop than those for the dopaminergic system. Nevertheless, they should be developed and will provide insight into the workings of other neurotransmitter systems. One of the reasons for the array of tracers available for the dopaminergic system is that drug companies have made a large number of compounds that interact with that system in their search for compounds of pharmaceutical interest. These have provided a valuable jump-

ing off point for the development of PET radioligands. An important role for labeled ligands for the neurotransmitter systems will be to support development of new therapeutic drugs. Competition experiments *in vivo* between known labeled compounds and new experimental drugs will provide new data on the transport, binding and relative retention of the new unlabelled compounds at the site of interest. This is demonstrated in the use of labeled MAO-B inhibitors by the Brookhaven group to measure the extent and time course of a new drug, RO 19 6327, whose mode of action was thought to be via MAO-B inhibition.[38]

Tracers to follow these highly specific neurotransmission processes have to meet a stringent set of requirements for specificity and sensitivity. A ligand for the dopamine receptor must be specific for that receptor and not interact with the serotonergic system or with related receptor subtypes. This is particularly demanding when the subtype of interest is present at significantly lower concentration than a competing subtype, because binding rate is a function of both the affinity of the ligand to the receptor and the concentration of each receptor subtype. Many of these ligands have structural features in common and often selectivity is achieved with fairly subtle changes in their molecular structure.

The sensitivity requirements may be even more demanding and fall into three categories. The first is that the tracer must be sensitive to the concentration of the binding site. This reduces to a requirement that the rate at which the radioligand binds to the receptor must be slower than its rate of delivery. If the rate of binding is more rapid than its rate of delivery and the concentration of the tracer is low compared to the binding site, then everything that is delivered will bind in one pass and the resulting image will not reflect the concentration of binding sites. Some radioligands have been developed that show beautiful images of receptor-rich tissue but in which the image reflects delivery more than receptor concentration. The labeled spiperone derivatives were cited earlier as examples of this.

The second sensitivity requirement is that the binding site for the tracer must truly reflect the biochemical process of interest in order to interpret the image. The information obtained from a fluoroDOPA study is largely the level of the enzyme that decarboxylates DOPA and fluoroDOPA, aromatic amino acid decarboxylase. However, the endogenous precursor in the synthesis of dopamine is tyrosine, which undergoes hydroxylation to DOPA by aromatic acid hydroxylase and this enzyme is the rate limiting step in dopamine synthesis. Thus the accumulation of [^{18}F]-fluoroDOPA in the brain is a measure of the decarboxylase activity, not the hydroxylase, and so is not that sensitive to the rate of dopamine synthesis. However modeling techniques have made it possible to extract useful information on dopamine synthesis rates from this tracer, even though the measurement is not being made at the most sensitive point. The third sensitivity requirement is that the process being measured must be sensitive to the pathology under investigation. All biochemical processes are regulated and most have spare capacity so that they can respond to abrupt changes in demand. If, in a region of interest, half of the dopaminergic neurons have died but the other half are compensating by producing twice as much dopamine, then any PET measurement of the rate of dopamine synthesis will give a value that is unchanged from a region with a full complement of neurons. Thus the measurement will be insensitive to the number of neurons, measuring only the amount of dopamine they are producing. This limitation is discussed in a recent review on tracers for the vesicular transport system[39] and emphasizes the complementarity of imaging the multiple aspects of neurotransmission.

The number of PET tracers that have been synthesized for brain studies runs into the hundreds. The number with interesting and useful properties is less, but still formidable. From this array of compounds will come some that are both sensitive and specific to individual processes in the central nervous system. It should be anticipated that the processes that are involved will be of specific relevance to both normal physiology and the departures that occur in disease states. PET radiopharmaceuticals will play an important role in expanding our understanding of the normal and diseased brain and in developing and evaluating new approaches to therapy.

HEART RADIOPHARMACEUTICALS

Among the earliest positron emission tomographic images ever made was that of a dog heart, imaged with [^{11}C]-palmitic acid.[40,41] The data was collected by rotating the dog between six sodium iodide detectors and demonstrated the feasibility of the PET method. The image quality was poor by

today's standards but our understanding of the meaning of the data implicit in this image of palmitic acid uptake has not changed that much. We didn't understand it then and, after a lot of research, we still don't fully understand it. The image is more closely related to blood flow than to oxidative metabolism, but not uniquely related to either.[42] The problems are almost certainly due to the mechanism of transport of the fatty acid from the carrier albumin to the mitochondria and the reversibility of the first step in the metabolism, the formation of the fatty acid-CoA derivative.

The first cardiac PET studies were directed towards the measurement of blood flow; metabolism was a secondary interest. A variety of agents have been tested and, as might be anticipated, the ones that gave the most valid data were the most difficult to use. Agents for measuring blood flow depend on the tracer being efficiently extracted from the blood into the tissue and this extraction being independent of blood flow. With knowledge of the tracer concentration in the blood, obtained by sampling the blood pool, and knowledge of the tracer concentration in the tissue, obtained from the PET image, blood flow can be calculated in units of volume of blood per unit time per volume of tissue. If the efficiency of extraction does not change in the organ being studied, then even without knowledge of the tracer blood concentration, the image will provide a relative distribution of blood flow in that organ. Thus the critical parameter for the successful tracer is that the tissue extraction be high, preferably 100%, and unchanging under the conditions that might be encountered. It is safe to say that no blood flow tracer meets these conditions but some approach it reasonably satisfactorily.

There are two broad divisions of blood flow tracers, those that are freely permeable and those that are trapped. The freely permeable tracers such as [^{15}O]-water wash into the tissue rapidly and then wash out again.[2] The trapped tracers, [^{13}N]-ammonia, [^{82}Rb]-Rb^+ and [^{38}K]-K^+ are transported in the tissue and stay there for sufficient time for the imaging. All of these tracers are highly extracted at physiologically normal blood flows but the extraction falls as the flow rises. The fall-off starts earlier and is more extreme for the cationic tracers than it is for the freely permeable ones.[1,43] Thus all the tracers will underestimate high flows, with the cationic species showing the greatest error. The freely permeable tracers are more difficult to image because the data have to be collected in a much shorter time frame and the concentration of the tracer in the blood remains similar to that in the tissue. Thus the blood in the chambers of the heart has very little contrast with the muscle of the heart. [^{82}Rb]-Rb^+, which is generator produced and has a half-life of 75 seconds, has the obvious convenience of not requiring a cyclotron, but it has a special set of limitations.[44] The extraction starts to fall off at lower flow rates than the other tracers and the short half-life imposes additional technical demands on the data collection. Thus if the purpose of the PET study is to gain a qualitative image of relative myocardial blood flow then [^{13}N]-ammonia is easy to make and is not particularly demanding on the PET imaging instrumentation. However, if accurate quantitative data is required then the additional benefits of an [^{15}O]-water study should be considered.

Measurements of cardiac metabolism have been made using several [^{11}C]-fatty acid derivatives,[45-47] [^{18}F]-2-deoxy-2-fluoro-D-glucose (FDG),[48] [^{15}O]-O_2[49] and [^{11}C]-acetate.[50] A simple static image of FDG provides beautiful cardiac images, but its value is greatly increased when compared with another tracer. Schelbert showed that [^{13}N]-ammonia could be used to measure reduced blood flow following myocardial infarction and that this reduced flow, accompanied by elevated FDG uptake, was a valuable tool in identifying viable tissue at risk.[1] The obvious explanation of this phenomena is that in regions of low blood flow, and therefore reduced oxygen supply, there is some anaerobic metabolism of glucose. Glucose is a much less efficient energy source in the absence of O_2, producing 3 mols of ATP per mol of glucose versus 36 mols from completely coupled oxidative metabolism of glucose; therefore more substrate is needed. The concept that increased FDG uptake in regions of decreased blood flow is due to anaerobic metabolism has proved hard to substantiate and is probably not the complete explanation.[51] Detailed examination of the relationship between the uptake of FDG in the heart and the cardiac utilization of glucose as a fuel for metabolism has shown that it is complicated. The heart is an omnivore and has a variety of metabolic fuels available; the differential affinity of glucose and FDG for the first enzyme in the metabolic process, hexokinase, is dependent on insulin.[51,52] Furthermore, the heart is constantly synthesizing and consuming glycogen, with no

clear relationship between the time a glucose molecule is incorporated into glycogen and when it comes out and is further metabolized.[53] All of these factors combine to make the relationship complex between FDG uptake and the use of glucose for production of ATP and metabolic work. The relationship is clear in a qualitative sense, and regional changes in FDG uptake in the heart are clearly indicative of regional changes in myocardial metabolism, but a true quantitative relationship between the two has proved elusive.[3]

Fatty acid images are even more difficult to interpret as there are problems with delivery and reversibility in the early metabolic steps. In addition, once the β-oxidation pathway has started, the process after the conversion of the two-carbon fragment to the acetyl CoA derivative is rapid and label leaves the tissue as carbon dioxide. There have been attempts to overcome this problem with fatty acid derivatives in which β-oxidation was blocked by the introduction of methyl groups[45-47] or a fluorine,[54] but the problems remain of reversible formation of the fatty acid-CoA derivatives and of delivery.

Oxygen-15 oxygen is the obvious tracer to measure cardiac energy use[55] because its metabolism is relatively free of the ambiguities presented by glucose and fatty acids, but it is technically challenging. The problem is that, although oxygen is freely diffusable, the product of oxidation is [^{15}O]-H_2O which is also freely diffusable. Thus any data analysis must account for the wash in and wash out of both the substrate for the reaction and the product of the reaction. This calls for high temporal resolution images from the tomograph and sophisticated modeling techniques, but it has been used successfully to image regional blood flow and oxygen consumption in the canine myocardium.[56] The method requires considerable expertise to use and could not be described as being ready for routine clinical use but the fact that its interpretation is unambiguous recommends it for definitive studies of cardiac energy metabolism.

Carbon-11 acetate has been used as a tracer to measure oxidative metabolism. With this compound washout of the tracer rather than the rate of uptake is the parameter of interest.[50] The theory is that acetate is a permeable intermediate in oxidative metabolism, regardless of the source of the fuel. Glucose and fatty acids both provide acetate that enters the TCA cycle, where it is oxidized to carbon dioxide and water with the release of energy in the form of ATP. Oxidative metabolism in the presence of [^{11}C]-acetate will result in the production of [^{11}C]-carbon dioxide which will wash out of the tissue. Thus the rate of washout of label is a function of the rate of turnover of the tricarboxylic acid cycle, provided that the turnover of the carbon dioxide pool is faster than the rate of production of carbon dioxide. This approach has been validated and the tracer appears to be promising for imaging regional rates of the tricarboxylic acid cycle within the heart, and thus measuring oxidative metabolism.[50,57-59]

Tracers for the neurohormonal systems that control the fundamental actions of the heart such as heart rate, contractility, conductance of the pacing signals, and vasodilation of the capillary system are under development but have proved significantly more difficult than anticipated. These systems have the same general structure and function as those in the brain, including synthesis, storage, release, receptor binding, re-uptake, and degradation of neurotransmitters. Typically there are two systems for each function that work in opposition; if the action of one system is stimulatory to a process then the action of the other will be inhibitory. Stimulation of the β-adrenergic system causes increased heart rate when there is a demand for increased blood supply. In chronic heart failure and following myocardial infarction the cardiac system shows a decrease in sensitivity to β-adrenergic agonists[60] and this has been shown to be at least partially a function of declining β-adrenergic receptors as well as a decline in the re-uptake efficiency of the neurotransmitter.

Several of the first labeled β-adrenergic antagonists failed to show receptor mediated binding *in vivo* although this had been demonstrated *in vitro*.[61] The first useful positron labeled β-adrenergic antagonist [^{11}C]-CGP 12177A,[62] the S isomer of the racemic ligand, shows receptor mediated binding *in vivo* and is selective for receptors on the cell surface but shows little selectivity for the β_1 and β_2 subtypes. The critical distinction between the sub types is that β_1 receptors respond to regional and β_2 to global stimulation. Modeling techniques have shown that [^{11}C]-CGP 12177 is maximally sensitive to the receptor concentration at a receptor coverage of around 50%; the sensitivity falls both at low levels and as the coverage approaches 100%.[63] This result demonstrates the need to con-

trol the amount of radioligand that is delivered to the receptor in order to provide accurate quantitation of the receptor. In the past the assumption has been that "high" specific activity is advantageous. In fact too high a value can be just as difficult to interpret as too low a value.

More recently the lipophillic β-antagonists, [^{18}F]-fluorocarazolol and [^{11}C]-carazolol have been prepared and evaluated.[64,65] These ligands are also unselective for the β-subtypes but they have access to both surface and internal receptors and it will be useful to compare the changes in the two receptor populations in both normal and disease states. The surface receptors are immediately available; internalized ones are a reserve that can be brought into play as needed.[66] The cardiac muscarinic receptors, which work in opposition to the adrenergic receptors, have also been imaged in a canine model with [^{11}C]-methyl quinuclidinyl benzylate (MQNB).[67] Sensitivity to the receptor concentration required 90% coverage of the receptor by the ligand and, once again, the tracer principle is probably in jeopardy.

The neurotransmitters for the adrenergic system are epinephrine and norepinephrine and two tracers for the pre-synaptic uptake of these neurotransmitters have been developed. [^{18}F]-Fluorometaraminol[68] and [^{11}C]-meta-hydroxyephedrine (MHED) have been synthesized by the Michigan group[69,70] with the objective of measuring changes in the re-uptake rates in situations in which the heart's sensitivity to catecholamine stimulation has declined. These include chronic heart failure, sudden death, depression that follows an MI, and aging.[71] Unfortunately the specific activity of the fluorinated compound was low because it was made using carrier-added fluorine gas. However, [^{11}C]-m-hydroxyephedrine has proved to be an effective tracer for the presynaptic adrenergic system, and modeling techniques have identified changes in this system with pharmacological intervention and disease.[72]

[^{18}F]-Fluorodopamine has been used as a tracer for the cardiac dopaminergic system.[73] This system is the stimulatory half of the vasodilation of the heart, adenosine being the other half. The compound is extensively and rapidly metabolized,[74] which complicates any modeling. It has not been widely evaluated although it has proved useful for answering some important basic questions about cardiac physiology, and simple estimation of regional differential uptake and wash out of the tracer has proved useful in a variety of cardiac diseases.[75]

The initial PET studies of the heart were focused on issues of metabolism and hemodynamics but PET is useful for answering many other questions about cardiac function. What will be the nature of the next generation of PET tracers for the heart? With increasing knowledge of the heart and its associated diseases it becomes clear that problems that on first analysis appear simple are a complex interaction of biochemical, physiological, and control issues. For example, obstruction in an artery that results in an inadequate blood supply for the cardiac tissue can initiate a complex series of changes, not only in oxidative metabolism but also in the efficiency of the systems that control conductance, heart rate, and contractility. Our current tracers paint their pictures with a broad brush but we can expect that this will change. Tracers for the β-adrenergic receptor will be sensitive to receptor concentration when used appropriately, and will eventually be selective for the different receptor subtypes and the different cellular locations of the receptor. Tracers for additional aspects of the sympathetic and parasympathetic nervous systems will be developed so that any change in the systems that control the function of the heart will be measured. As our understanding of the biology grows, the selectivity of the tracers will grow along with their sensitivity to measure the biochemistry that is under investigation.

RADIOPHARMACEUTICALS FOR CANCER

Just as Warburg's hypothesis of aerobic glycolysis[76] dominated studies of cancer biochemistry for two decades early in this century, PET with ^{18}FDG has dominated metabolic imaging studies of cancer during the last two decades. But studies of cancer biochemistry have gone far beyond the original theory that cancer cells originate from normal cells as a result of irreversible injury to respiration. Is the association of glycolysis with growth rate secondary to loss of control of growth and replication? New techniques in molecular biology allow us to study the relative autonomy of cancer cells from a chemical perspective. Cellular control can be studied at the level of errors in DNA. This new knowledge of cancer biochemistry is leading to the promise of improvements in therapy. The challenge is to apply this knowledge to design useful PET radiopharmaceuticals. PET should not be limited to

simply hunting for disease. Its strength is in providing unique information on the biochemistry of disease, specifically information that can be used for the benefit of the patient.

How can metabolic imaging with PET contribute to our understanding of cancer biology? Will this information provide more effective treatment of the individual with cancer? Cancer is a multifaceted disease process, often involving loss of regulation, abnormal metabolism of substrates, changes in cellular membranes, and increased and uncontrolled growth and proliferation of cells, resulting in biochemistry distinct from mature and healthy cells. As we gain knowledge of the diversity and differences of tumors, laboratory science provides rationales to support more experimental treatment strategies than could possibly be evaluated by well-designed prospective clinical trials. When a new treatment strategy is proposed, the most difficult task for the oncologist is selecting which patients will benefit from new concepts.[77] Allied with this choice is the common clinical dilemma: if the randomized trial for a new treatment gives similar control rates as for conventional therapy, are there different subpopulations failing the different treatment regimens? If this is so, how can these subpopulations be identified? Alternatively, predictive assays of specific causes of resistance to therapy[78,79] would permit rational selection of patients for specific therapy, without including those who had no hope of benefiting from that approach. Metabolic images of regional tumor biochemistry and physiology done before, during, and long after therapy are helping us learn to use imaging to select appropriate therapy for the individual patient. Several important questions can be addressed:

- Can we use metabolic imaging to select the best treatment?
- Is the patient benefiting from a course of therapy or should it be abandoned in favor of an alternative scheme?
- Are healthy tissues being damaged too severely by a treatment?
- When has disease been controlled? Is there evidence of local recurrence?
- Are cells receiving an adequate supply of nutrients and drugs?
- How much, and by what pathways, are they using these nutrients?
- Are there proliferating cells, and how is proliferation altered by treatment?

Many decisions by the clinical oncologist currently rely on invasive sampling at limited tumor sites and times during the course of therapy. PET has the advantage of providing information from the whole body and can provide many useful answers to questions that oncologists ask.

FDG/PET is a valuable tool that provides useful metabolic imaging information complementary to anatomical imaging and that supports oncologists in the most effective care of their patients. In fact, several laboratories are now developing electronic techniques that overlay metabolic images of FDG with anatomic images from MRI or computed tomography (CT). Wahl has suggested the term "anatometabolic" images as a proposal to combine the best of anatomic and of functional image resolution.[80] However, radiopharmaceuticals are also being developed that characterize tumor biochemistry more specifically than can be done with ^{18}FDG. Some studies look further into the deep levels of cellular energetics, beyond the hexokinase reaction; other radiopharmaceuticals measure tumor growth at the level of DNA replication and protein synthesis. The hormonal status of tumors is being characterized by PET imaging, as are factors such as hypoxia that influence response of a tumor to therapy. PET is being used to observe the pharmacokinetics of drugs, including antibodies. In each case these radiopharmaceuticals are intended to study metabolic differences between normal, malignant, and treated tissue and to determine how these differences might lead to more useful strategies for differentiating tissues based on biochemical characteristics.

Glucose metabolism reflects cellular energetics in tumors. The pioneering work of Som et al[81] on the use of FDG for tumor detection has led to one of the great success stories of nuclear medicine. The value of ^{18}FDG for staging tumors and differentiating recurrence from fibrosis is widely accepted.[82]

Nonetheless, cancer is a disease of altered enzymology associated with loss of regulation of growth and some of the assumptions required for inferring glucose metabolic rate from observations of ^{18}FDG may not be warranted. Our group has compared ^{18}FDG and 1-[^{11}C]-glucose in tumors, with the conclusion that the ratio of the MR_{FDG} image to the MR_{Glc} image, a regional measure of the apparent lumped constant, for human glioma is variable and much greater than that of normal brain.[16] This

observation leads one to question the extent to which differences between normal brain and tumor glycolysis contribute to lowering the calculated MRGlc through rapid lactate formation.[83] There is also growing evidence that some tumors acquire much of their energy from mitochondrial metabolism of substrates not directly involved in the TCA cycle, but that supply intermediates to it. Labeling of such substrates could provide important new information on tumor metabolism and how it changes with therapy.

The motivation to develop radiopharmaceuticals for imaging cellular growth derives from the tenet that the cell's biosynthetic machinery, rather than its fueling processes, is most fragile toward cytotoxic agents. Damage to chromosomal DNA renders clonogenic death, but can leave surviving mitochondria that continue to metabolize energy substrates. To this end, [^{11}C]-thymidine (TdR) was developed as a specific marker of DNA synthesis. An advantage to this approach is that clearer pictures of residual disease after therapy should be realized, uncomplicated by the problems with FDG accumulation in inflammation or in healthy cells invading the original tumor site.[84,85] Limitations of [^{11}C]-TdR, however, stem from its rapid degradation, which competes with DNA synthesis. To better isolate global metabolism of labeled nucleosides towards DNA synthesis, our laboratory is developing TdR mimics such as [^{11}C]-AZT[86] and [^{18}F]-3′-deoxy-3′-fluorothymidine (FLT),[87] which enter the DNA synthetic pathway and are stable to systemic degradation.

Tracers of protein synthesis have also been advanced as markers of biosynthesis. Several labeled amino acids have been proposed, although none have been thoroughly validated for measuring protein synthesis. The methods using labeled amino acids are complex, with many potential pitfalls. Perhaps the most widely used amino acid radiopharmaceutical is [methyl-^{11}C]-methionine, but its biochemistry is among the more complex of the natural amino acids, involving transmethylation pathways as well as biosynthesis. The general limitation to measuring protein synthesis from amino acids stems from their secondary metabolism[88] and their dilution into unknown pool sizes of intracellular amino acids and their acylated tRNA's. This is exacerbated by differences in local protein reutilization. A fresh approach to protein synthesis would be to image active ribosomes, the organelles responsible for protein synthesis, or to use labeled chain-terminating reagents as PET radiopharmaceuticals.

Yet another approach to imaging growth in cancer is based on the specificity of the Watson-Crick base-pairing formation. Antisense deoxyoligonucleotides have been used to block the expression of oncogenes in cancer cells. Transformed cells which express a specific oncogene are potential targets for radiolabeled antisense deoxyoligonucleotides specific to the oncogene. There appear to be some formidable obstacles to this line of research. Just one antisense molecule in the cell is sufficient to eventually knock down the expression on an oncogene, a therapeutic effect, but imaging will require a higher concentration of antisense molecules inside the targeted cells.[89] Thus successful development of antisense radiopharmaceuticals is a high-risk venture.

Imaging the presence and extent of estrogen (ER) and progesterone (PR) receptors should be useful in predicting the hormonal response of breast tumors, particularly of metastases, and in monitoring response to hormonal therapy. The ability of PET to evaluate ER in human breast cancer has been demonstrated with 16α-[^{18}F]-fluoroestradiol, and imaging showed a good correlation with ER content in tissue samples.[90] Alternative radioligands might have even better characteristics.[91] Labeled tamoxifen has been suggested as an imaging agent and has some attractive characteristics with respect to metabolism and persistence in the circulation.[92] However, the ideal ER compound has not yet been identified. Some very promising animal distribution results with labeled progesterones were not predictive of human studies, which were plagued by extensive metabolism.[93,94] At this time, there is no satisfactory agent for imaging the PR in humans, but the importance of this receptor in selecting and evaluating therapy justifies additional research to design a more stable compound.

Several neuroreceptor radioligands have also been evaluated for imaging receptors associated with tumors. None of these receptors are unique to the transformed cells, or are they apparently significant to the tumor's biology or therapy. Carbon-11 N-methylspiperone has been used to image dopamine sites in pituitary adenomas.[95] Tumors that contain this receptor respond to bromocryptine therapy and do not require surgery. Benzodiazepine ligands have been used to image PK-type binding sites in brain tumors. Glial cells have a high

concentration of this receptor, but imaging studies with several ligands generally found less uptake in tumors than in normal brain.[96] Further studies of imaging this receptor require better understanding of its role in cancer, and more specific PK radioligands.

^{11}C-Meta-hydroxyephedrine, a radiotracer developed to image norepinephrine reuptake in the sympathetic nervous system, has been evaluated for imaging pheochromocytomas[97] and neuroblastomas.[98] It is rapidly taken up and retained by tumors. As an imaging agent, it has high sensitivity and specificity for detection of neuroendocrine tumors; however, its value is unique to this tumor type and doesn't provide any characterization of the cancer beyond visualization of its location. Lastly, the recent and perhaps serendipitous finding of sigma receptors associated with breast and other tumors might result in a useful PET procedure,[99] although the specificity and functional significance of this receptor is not yet defined.

A variety of therapeutic approaches have been aimed at overcoming the cure-limiting effect of hypoxic cells in patients receiving radiation therapy, but with no clear therapeutic advantage. Because hypoxia may limit response in only a subset of patients, a convenient regional measure of hypoxia would be useful in individualizing therapy. Our laboratory has developed [^{18}F]-fluoromisonidazole, which accumulates in cells with low O_2 concentration, for imaging the oxygenation status of tumors.[100] Patients with positive images should benefit from therapy aimed at overcoming hypoxia, for example high LET radiation. Analogs of FMISO in which the reduction potential is changed so that the compound is reduced at higher oxygen levels, would permit imaging of hypoxia at a higher level of O_2 than the range where FMISO is trapped. Further development of nitroimidazole analogs should minimize alternative metabolic pathways of the compound so that binding is more purely reflective of regional O_2 levels.

A novel alternative approach to imaging hypoxia is being evaluated by Fujibayashi et al.[101] The method does not rely on the nitroimidazole structure. They have prepared a ^{62}Cu complex (Cu-ASTM) that is highly permeable and is easily reduced and retained in hypoxia. Their studies have focused on myocardial ischemia/hypoxia but this work shows that hypoxia imaging is a relatively undeveloped field with widespread significance in tumors as well as myocardial ischemia and stroke and potentially in imaging arthritis and wound healing.

Radiopharmaceuticals can also be used to monitor drug pharmacokinetics, although these procedures do not generally contribute to biochemical characterization of the tumor as much as to studying the pharmacology and catabolism of drugs. PET may also find a role in antibody imaging. Several radionuclides not used for metabolic imaging have been suggested: ^{89}Zr, ^{86}Y, ^{124}I, and ^{64}Cu, as well as ^{18}F, but their clinical value remains unclear.

In summary, the challenge to the PET community of chemists, biologists, physiologists, biochemists, pharmacologists and physicians, is to apply new knowledge of cancer biochemistry for developing and validating useful PET radiopharmaceuticals which will in turn produce useful procedures. Initially the synthesis of a compound containing these short-lived radionuclides was a triumph in itself. However as the science advances, the synthesis becomes just the first step in a long trail that terminates in the compound being used to provide data on a biological process when used in a well-designed and executed PET experiment. The resulting list of compounds and experiments will be as diverse as the biological system itself.

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