

Measurements of Biochemical Reactions In Vivo

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1.1

Introduction

Immense progress has been made over the last century in medical diagnosis, especially in the techniques of radiological imaging. The evolution began in the mid-1890s by the epochal discoveries of Röntgen and Becquerel (Blaufox 1996). The initial limitation of radiol-

ogy to observing externally radio-opaque structures such as bones in the living body rapidly evolved into functional imaging of organs such as kidney and liver and the circulatory system using radio-opaque contrast agents. With the advent of computed tomography soft tissues also became visible in high-resolution images, augmenting the spectrum of function studies of organ segments. Yet, the molecular-atomic level of biological organization in living tissues became observable only with the introduction of the radionuclide tracer technique by Hevesy (1913). Further studies with radioactive and stable isotopes of common elements in organic compounds (C, H, O, N, S, P) in the 1930s and 1940s revealed the surprising extent to which both “functional” and “structural” compounds in the body are ceaselessly formed and broken down, even with no change in overall amount, form, or function (Schoenheimer 1946; Hevesy 1948). Today, the specialty of nuclear medicine is indispensable in clinical diagnosis and therapy.

The current literature on nuclear medicine shows the wide usefulness of radionuclides as tracers of constantly occurring “life events” in the body, encompassing gross substrate turnover, blood perfusion, particular metabolic changes, and certain biochemical reactions at large or at defined sites. In fact, the tracer techniques have opened totally new insights into normal and pathologically altered cell functions in the living body, its organs and tissue segments. The technical advances that were needed to reach the present state of art required interdisciplinary efforts often on a relatively large scale. The powerful service to clinical medicine afforded by nuclear medicine is seen in: the introduction of useful, mainly gamma-emitting, radionuclides and of radiopharmaceutical chemistry for proper substrate labeling; the development of counting and imaging devices optimal for the task at hand; the burgeoning growth of data analysis with the help of models applicable to arrive at

meaningful diagnostic information and the correlated radiation dosimetry and biology (Wagner et al. 1995).

Observing *in vivo* accumulation or kinetics of radionuclides in the whole body or certain regions of interest (ROI) demands that radiation is registered preferentially by dynamic modes. The instruments are single- or multiple-counting probe devices, planar gamma cameras, single-photon-emission tomographs (SPECT) or positron-emission tomographs (PET), as discussed in Chap. 6. The suitable radionuclides must emit gamma radiations or be positron emitters. *In vitro* analyses of radioactivity in samples of blood or breath and/or body excreta such as urine or saliva at times complement data obtained by imaging or counting, or are by themselves useful in evaluating biochemistry. Such *in vitro* assays use mainly beta-emitting radionuclides to be analyzed, for instance, by scintillation counting, or by autoradiography of isolated cells, tissue sections, or chromatograms. Special *in vitro* methods of great importance are the radioimmunoassays, particularly to clinical medicine. For both *in vivo* imaging and *in vitro* assays, stable isotopes also must be considered for use with magnetic resonance imaging (MRI) and spectroscopy (MRS) and for metabolic studies with labeled substrates (see Chaps. 12–14). Sometimes in conjunction with MRI, MRS can observe and quantify certain kinds and numbers of biochemical reactions *in vivo*. *In vivo* and *in vitro* techniques with stable isotopes may be limited by physical constraints imposed by the signal-to-target-atom ratio. In principle, the radionuclide tracer method allows the best overall observation of any substrate undisturbed in its system environment by imaging of defined sites in the living body, as well as by *in vitro* assays, as long as radiopharmaceutical chemistry allows the substrate to be labeled appropriately. Indeed, choice, production, testing, and supply of tracer-labeled pharmaceuticals suitable as indicators appear to be essential for the success of nuclear medicine in assessing biochemical reactions in the intact living body (see Chap. 5).

Over the past few decades, interest has intensified in observing biochemical reactions, not only qualitatively but also quantitatively, as they occur *in vivo*. The goal has become not only to localize the biochemical events to certain regions in the body but also to express them in a manner that is conventionally expected from *in vitro* laboratory methods. *In vivo* quantification considers the controlling networks of biochemical reactions as they respond to fluctuating inputs of nutrients and various environmental

factors, which then also affect gene expression. The biochemical networks operate in patterns of mutual responses for the purpose of defense against, or adaptation to, challenges posed to the organism endogenously or from the environment. Defensive or adaptive responses of the organism occur at its molecular and cellular levels of organization and are principally triggered by cascades of molecular signals of different kinds not often easily understood from observations on isolated cells and tissue samples. A ready example is found in the operation of the immune system. The approach to quantitatively measure specific biochemical reactions as they occur *in vivo* brings new diagnostic information. It also opens new avenues to apply radionuclides as radiation sources for treating a variety of diseases at the level of single cells and molecules.

Quantification of single and/or multiple biochemical reactions is even more desirable in view of the nearly fully sequenced human genome; indeed, this development poses particular challenges to clinical medicine. Without doubt, gene expression patterns as they become increasingly available, for instance by using DNA array chips, are by themselves often insufficient for meaningful diagnostic revelation; they must be seen in the context of the whole biological system within its signaling network. Links between the genotype and phenotype need to be established *in vivo* at the level of molecular organization. This demands a close cooperation, even fusion, between molecular and cell biology, on the one hand, and the field of imaging techniques including radiopharmaceutical chemistry, on the other.

In the simplest case of one gene coding for one protein, the activity of one enzyme may depend upon the nature and integrity of one gene. However, most genes carry codes for various proteins with different functions. The diversities of genotypes and phenotypes are based on varying polymorphisms in individual genes, on a still largely elusive interplay between genes, and on regulatory mechanisms acting between gene products. Controls to be considered also are “epigenetic” phenomena, which involve changes in nuclear histones such as acetylation, methylation, phosphorylation, and appear, in addition to DNA-binding transcription factors, to govern the expression of particular genes. Post-translational alterations of proteins such as by glycation, methylation, and phosphorylation add to governing phenotypic diversity, as they may cause or be consequences of disease. Many clinical symptoms and findings re-

quire explanations at both the genome and proteome level.

Two examples of genetically determined or strongly influenced diseases are cystic fibrosis and diabetes mellitus. Cystic fibrosis will appear in about 1 of 2,400 newborn white children and in about 1 of 17,000 babies in the black population. This disease appears to be the consequence of changes in one known gene on chromosome 6 coding for the chloride channel protein. This gene has at least 350 known single nucleotide changes, or mutations (of which only a few, perhaps 7 or 8, seem to affect the clinical expression in terms of extent or location of pathology, be it in intestinal organs or lungs), mainly involving nearly all exocrine glands. Appropriate choices of nuclear medical tests may help to classify the disease (see Chaps. 2, 12). Type II diabetes has a much more complex genetic etiology, with at least 9 genetic allelic types of the insulin gene and 40 or more of the insulin receptor genes – and probably many more genes are also involved in causing this type of diabetes, yet to be discovered (see Chaps. 2, 14, 24). The ubiquity of insulin receptors in several organs suggests the possibility of genetically caused differences among them and the need for definition of multiple disease parameters, of which many may be evaluated by nuclear medicine procedures.

Most functional interactions between genes are little understood. To make these crucial reactions part of advanced diagnostic goals for eventual medical application, observations must include the intact signaling networks of living tissues *in vivo*. Any *in vitro* measurement on tissues, obtained for instance by biopsy, is *a priori* hampered by the fact that the physiological signaling within the complex system is practically excluded.

Thus, it appears timely to link *in vivo* biochemical reactions and reaction loops to genes. This may begin with a known pattern of expression of a defined set of genes in order to search for complementary downstream metabolic reactions. On the other hand, measured biochemical reactions, cell responses, and even tissue functions and local perfusion, may be the starting point, expressing a defined phenotype for the analysis of one or a set of known and unknown genes, an approach to what might be called “reverse genetics” (Wagner et al. 1995). In either approach, interrelationships between genes and metabolism are seen in the context of system cooperation under homeostatic control within physiologically functional networks or under pathologically operating condi-

tions. Moreover, such systems may be studied under conditions of an intentional disturbance. The latter may then help in defining the tolerance of the system to stress.

The radionuclide and radiopharmaceutical application and measurement techniques have matured to an astonishing degree of precision and accuracy so that they will eventually offer *in vivo* assessment of what is generally called “functional genomics and proteomics” in clinical medicine. Here, even relatively minute changes in reaction kinetics, or phenotypically defined physiological responses, need to be monitored *in vivo* so that individual changes of a biochemical reaction rate or of the balance between functionally related reaction rates become informative. In order to reach that goal, though, a number of factors that complicate quantitative measurements require attention.

This chapter aims at giving a condensed overview and introduction to various potentially useful approaches to quantitatively measure biochemical reactions *in vivo* in such a way that the data may eventually be linked to gene expression, secondary control mechanisms, and individual physiological parameters. The emphasis is on summarizing relevant advances to assess biochemical reactions *in vivo*, so that an understanding may be gained of gene-orchestrated biochemical and physiological functions in complex adaptive systems. It is clear that the attempt is far from being comprehensive and the task of understanding larger complex system functions appears overwhelming. Yet, the time has come to try to do it.

1.2

Substrate/Ligand and the Corresponding Target Molecule

For dealing principally with the various technical approaches to *in vivo* measurements of biochemical reactions, it is necessary to dwell briefly on the fundamental relationship between substrate/ligand and the corresponding target molecule. Any biochemical reaction brings molecules into close contact with each other. The interaction of a given substrate with its target enzyme or receptor depends on specific molecular configurations, so that both comply with the demands for intermolecular bonds to initiate a biochemical reaction. The ubiquitous device of “lock-and-key” as the fundamental fine biochemical struc-

ture for interaction of compounds in metabolism, cell signaling, etc., was first articulated by Emil Fisher in 1890. Later, in 1952, this model was extended by Daniel Koshland to include the concept of a “molded fit” between compounds, e.g., a ligand or substrate and its target or receptor protein (Stryer 1995). Although the occurrence of either rigid “lock-and-key” or more flexible “molded fit” in any particular instance may be infrequently recognized, such concepts could be important considerations in nuclear medicine tactics and interpretation.

The molecular configuration of the target or receptor protein such as enzymes and cell surface receptors determines the accommodation of the labeled substrate or ligand. Thus, binding of substrate or ligand depends on the ultimate third-order structure of the target compound, which in turn may vary according to certain polymorphisms of the gene or genes that direct the structure. This may express different phenotypes that govern binding of the labeled compound. The concept applies importantly as well to drugs as ligands and to the individuality of response to drugs. Adaptive changes in the molecular configuration of the target protein come through various additional molecular interactions with the target and may, for instance, involve reaction products in feedback loops affecting enzyme-catalyzed reactions, or involve small molecular effectors, such as Ca, Na, K, Mg, nitrous oxide (NO), and reactive oxygen species (ROS). These “signaling” molecules and ions may also be observed by applying the tracer technique. The ensuing controls affect the rate of a reaction with the primary substrate in various categories of biochemical reactions, be they receptor-ligand interactions, transport functions, or enzyme-catalyzed reactions. In addition, corresponding up- or down-regulation of a reaction may be brought about by altered numbers of available functional target molecules, such as receptors at cell membranes.

Moreover, slight changes in substrate configuration, which may occur for instance through radionuclide labeling, may significantly alter the rate of interaction between substrate and target, and subsequently influence the biochemical reaction. Intuitively, the “molded fit” model might permit binding of a tracer substrate to the appropriate target more variably different from the natural substrate than would be accommodated by a rigid “lock-and-key” case. This could allow for greater latitude of competition between the labeled substrate and its natural counterpart. The degree to which a labeled and often thereby unnatural analogue of a sub-

strate fits the target mold could be approximately proportional to its binding affinity.

The interaction between substrate and target molecule may cause the substrate to remain bound to its target for a comparatively prolonged period of time; such is often the case in ligand-receptor interactions. The latter are essential again for activating biochemical reactions through changes of molecular target structures that are linked within the cell downstream from the receptor (see Chap. 4). A substrate may be bound to its target in order to be transported, for instance, across a cell membrane using a molecular channel system. Such active transport is common for most metabolic substrates. A substrate that binds to its target enzyme usually experiences a change in structure, either by molecular rearrangement, cleavage in some form, or by addition of a molecular subgroup. Some substrates undergo a sequence of such reactions. Enzyme-catalyzed reactions determine the vector of metabolic activity in the sense of substrate synthesis or catabolism.

For a substrate to meet its target at the reaction site, it may be synthesized on site, or diffuse to it, or be transported to it from the circulating blood through the interstitial space into the cell, or it may come from a neighbor cell or interstitial space. Within the cell, the substrate must pass through cellular compartments in order to reach its reaction site. These factors complicate in vivo measurements, as discussed later in this chapter.

1.3

Goal of Measurement

Enzyme-substrate interactions were recognized from early times as underlying a successive series of chemical changes in a substrate, for instance, from ingested food. This process called “metabolism” broadly encompasses either molecular breakdown, catabolism, or synthesis, anabolism. Metabolic changes can also be closely associated with transport across a cell membrane. Enzymes and transporting proteins appear to act in concert with other enzymes toward a physiological “goal,” but as individual proteins may be separate from other related enzymes in a system and may have individual fates and integrity. Radionuclide tracers have helped to establish types and rates of biochemical reactions in functional circuits.

Those who measure biochemical reactions in the body may resort to selecting one or more specific labeled substrates for in vivo imaging or counting, or for in vitro analysis of tracer in exhaled breath, body fluids, or excreta. In the former approach, one explores at a selected ROI the rate and degree or amount of accumulation, or rate of disappearance of a labeled substrate either alone or in conjunction with an internal standard, as explained below. The latter approach determines the rate of appearance of one or more labeled reaction products in the breath, urine, or saliva of the patient consequent to metabolism of the labeled substrate through a known enzyme.

Ideally, the final outcome of a measurement of a biochemical reaction in vivo should be comparable to the data obtained by in vitro testing by conventional analytical procedures. In other words, the key parameters that conventionally describe a biochemical reaction are also the optimal goals for nuclear medicine approaches. A less ambitious task, but presently widely accepted in the practice of medicine, is simply the proof that a reaction occurs in the body or in a given ROI. This may result in a semi-quantitative statement regarding the degree of some increments by which a labeled substrate or ligand binds to its target molecule or undergoes a structural change perhaps associated with the release of a labeled reaction product. But for eventual assessment of consequences, for instance, of polymorphism-related functional changes of a target protein in a biochemical reaction, the measurement must be sensitive, specific, and quantitative.

In order to plan the measurement to be as quantitative as possible, the investigator should at the outset be familiar at least with first-order kinetics of biochemical reactions. These are well explained in biochemistry textbooks (Stryer 1995; Rodwell 1996). Thus, the Michaelis-Menten treatment of a biochemical reaction also appears to be a requirement for data measured in vivo. It leads to an understanding of reaction rates, their constants, and the velocity of a reaction as it depends on substrate concentration in relation to the available amount of enzyme (and similarly of receptor or transporter). In short, with:

E = total amount of enzyme available

S = substrate concentration

ES = amount of enzyme bound to substrate

k_1 = rate constant of substrate binding to enzyme

k_2 = rate constant of separation of substrate from enzyme

k_3 = rate constant of enzyme catalyzed reaction of substrate

then:

$$(E - [ES]) \cdot S \cdot k_1 = [ES] \cdot k_2 + [ES] \cdot k_3 \quad (1.1)$$

or by rearrangement:

$$\frac{E \cdot S - [ES] \cdot S}{[ES]} = \frac{k_2 + k_3}{k_1} \quad (1.2)$$

or

$$\frac{E \cdot S}{[ES]} - S = \frac{k_2 + k_3}{k_1} = K_m \quad (1.3)$$

K_m is the Michaelis-Menten constant.

If all E is involved in the reaction, the reaction has its maximal velocity V_{\max} :

$$E \cdot k_1 = V_{\max} \quad (1.4)$$

If only $(E - [ES])$ or $[ES]$ alone is involved in the reaction, the reaction velocity is v ; let only $[ES]$ be involved:

$$[ES] \cdot k_1 = v \quad (1.5)$$

Inserting Eqs. 1.4 and 1.5 into Eq. 1.3:

$$\frac{V_{\max} \cdot S}{k_1 \cdot v/k_1} - S = K_m \quad (1.6)$$

or

$$\frac{V_{\max} \cdot S}{v} = K_m + S \quad (1.7)$$

and

$$v = \frac{V_{\max} \cdot S}{K_m + S} \quad (1.8)$$

This then states that the velocity v in a first-order reaction is proportional to substrate concentration S and to $V_{\max}/(K_m + S)$, i.e., with increasing S , the value of v asymptotically reaches a plateau at V_{\max} when S becomes near equal to $(K_m + S)$. The expression $V_{\max}/(K_m + S)$ in Eq. 1.8, for a given value of S , is a rate constant, which declines with increasing substrate concentration.

It is obvious that K_m relates to a value of S . In order to determine the K_m in terms of S , Eq. 1.8 is rearranged:

$$K_m = \frac{V_{\max}}{v} \cdot S - S$$

It is clear that in the case of v being one half the value of V_{\max} :

$$K_m = 2S' - S' = S' \quad (1.9)$$

Thus, K_m is equal to the substrate concentration S' at v being one half of V_{\max} .

The values of K_m and V_{\max} can be easily obtained graphically by the reciprocal expression of Eq. 1.8:

$$\frac{1}{v} = \frac{K_m + S}{V_{\max} \cdot S}$$

or:

$$\frac{1}{v} = \frac{1}{V_{\max}} + \frac{K_m}{V_{\max}} \cdot \frac{1}{S} \quad (1.10)$$

Equation 1.10 is the Lineweaver-Burke function. It gives V_{\max} and K_m from plotting the reciprocal of the measured velocities v of a reaction against the reciprocal of different substrate concentrations S , at which the measurements were made. The resulting straight line intercepts the ordinate at $1/V_{\max}$ and has the slope K_m/V_{\max} .

It follows that the optimal goal of measurement of a biochemical reaction in vivo, if applicable, should be the determination of rate constants at different substrate concentrations, and subsequently K_m and V_{\max} .

Rate constants may apply to different types of substrate transitions that comply with first-order reaction kinetics. Such transitions also pertain to compartmental analyses. The corresponding rate constants k individually conform to the differential equation $dC(t)/dt = k \cdot C(t)$, with $dC(t)$ giving the incremental change of radioactivity at time t during the time increment dt .

Indeed, as will be shown in more detail below, measurements on tracer movement in and out of a ROI can readily provide the inputs needed for compartmental analyses. Thus, in a simple one-tissue compartment model, the transit of an indicator from the circulating blood into the free substrate pool in tissue, and its return from there to the blood can be described by the clearance of tracer from the blood and the rate constant of tracer return to blood. An example of the application

of a one-tissue compartment model describes the transport of glucose across the blood-brain barrier. The labeled glucose analogue 3-methyl-glucose is here the indicator. It is not metabolized but returns from the free glucose pool in the extravascular tissue space, mainly in glial cells, back into the circulating blood. The inflow of this indicator from the capillary flow system across the blood-brain barrier into the glial cells is given by K_1 , which expresses the clearance rate. The indicator outflow back from the glial cells into the circulating blood adheres to the rate constant k_2 . The corresponding expression is:

$$dC_T(t)/dt = K_1 \cdot C_p(t) - k_2 \cdot C_T(t)$$

with $C_p(t)$ being the radioactivity of indicator in the circulating blood plasma in counts per unit volume at time t and $C_T(t)$ the corresponding radioactivity in the extravascular tissue space at time t . It is to be noted that the ratio of amounts of indicator to glucose is different in blood and extravascular tissue space; in contrast to its indicator, glucose continues to be metabolized. This is important, because the value of k_2 here also depends on the amount of free glucose within the extravascular compartment, which varies with the extent of glucose metabolism.

A two-tissue compartment model applies when the indicator after entrance in to the tissue binds to a target molecule from where it again may separate intact or after a catabolic or anabolic alteration. In this instance, the clearance rate of the indicator from the blood into the tissue is conventionally designated k_1 , the rate of return from tissue to blood is k_2 , the acceptance rate of the labeled compound by its target molecule is k_3 , and the rate of dissociation from this bond is k_4 ; in addition, the rate of biochemical generation of a labeled product that would transfer into the circulating blood may be k_5 (see Chaps. 7, 8).

Widely used in clinical practice today is the measurement of phosphorylation of glucose by using as indicator the labeled glucose analogue 5-deoxyglucose (Sokoloff et al. 1977). This glucose analogue when phosphorylated fails to be accepted into the next physiological metabolic reaction. It accumulates at the site of the primary phosphorylation. Thus, 5-deoxyglucose when labeled with ^{18}F allows in vivo quantification of glucose phosphorylation by measuring (with PET) the irreversible tracer binding, be it in brain, heart, or malignant tumor (Gallagher et al. 1977; Phelps et al. 1979; Reivich et al. 1979; Delbeke et al. 2002). In this instance, the rate constants k_4 and

k_5 in the two-tissue compartment model are practically irrelevant. The applicable equations relate to the two-tissue compartments: free tissue pool C_{TF} and the substrate binding site C_{TM} :

- 1) $dC_{TF}(t)/dt = K_1 \cdot C_P(t) - k_2 \cdot C_{TF}(t) - k_3 \cdot C_{TF}(t)$
- 2) $dC_{TM}(t)/dt = k_3 \cdot C_{TF}(t)$

with $C_{TF}(t)$ being the radioactivity of indicator in the free tissue pool in the ROI at time t ; $C_{TM}(t)$ the radioactivity of indicator bonded at the reaction site in the ROI at time t , and $C_P(t)$ the radioactivity of indicator in arterial blood plasma at time t .

In a similar fashion, the practically irreversible binding of a labeled ligand to its receptor is readily accessible to compartmental analysis. More details appear later in this chapter and especially in Chap. 7.

From the above it is also clear that the measurement of certain reactions may well rely only on one or more rate constants without need to quantify substrate concentration. The advantage of restricting observation to the rate constants is the easy availability of measuring devices such as SPECT, that do not readily allow quantification of labeled substrate as PET does. Thus, biochemical measurements are open to anyone who has the conventional nuclear medical imaging devices that permit the dynamic mode of data registration.

1.4

Factors that Complicate Measurements of Biochemical Reactions

The quantitative measurement of a biochemical reaction *in vivo* using the tracer technique requires attention to various factors that potentially complicate the interpretation of measured data. Of course, the amount of indicator must be in the tracer range, so as not to preclude the observation of the wanted reaction. Moreover, external measurements of tracer only relay the fate of the tracer, such as a gamma-emitting radionuclide, and not necessarily that of the compound labeled as indicator to which the tracer is attached. Only if it is certain that the tracer remains bound to the labeled substance during the time of observation, does the measurement represent the indicator. On the other hand, separation of tracer from the indicator may testify to the occurrence of a biochemical reaction.

In the context of ROI imaging or counting, various factors need consideration that influence, for example,

the path of the indicator in the body on its way to its molecular target within the ROI. The indicator is administered to the body usually by intravenous injection. Alternative routes are intraarterial, intracavitary, and interstitial injections, as well as ingestion and inhalation. Prior to reaching its target, the indicator is usually transported via the blood circulation into the target tissue, where local blood flow is an essential factor in indicator distribution. Within the organ, transport from the blood vessels into the interstitial space and diffusion through interstitial space, even if usually a fast process, may find barriers to the indicator's reaching the target cells for external binding. After having been bound, the indicator must be internalized to reach its intracellular target molecule. Finally, conditions within the target-containing cells may affect the final destination of the indicator. Similarly, measurements of tracer in body excreta demand attention to the kinetics of the labeled reaction product until it appears in the excreta. One needs to account for the various influences on measured indicator and tracer kinetics in order to quantitatively evaluate the kinetics of the wanted reaction.

An illustrative example is the imaging of tracer accumulation in a given ROI, under conditions of stability of tracer on the indicator. The kinetics of indicator inflow is supposed to signal indicator transport or binding to its receptor or enzyme, and the outflow rate should express either indicator release or a biochemical reaction with liberation of tracer. In any case, clearly, the chosen indicator must be appropriately labeled. Serial measurements must use an optimal image device such as a scintillation camera in the planar or tomographic mode and should begin right after the injection to register incremental tracer inflow, accumulation, and outflow in a chosen ROI. This dynamic imaging may be omitted in favor of a still image if it is properly timed after injection and if the indicator can be quantified on site, such as by PET (as explained in Chap. 7). This delayed imaging may help to minimize the influence of local blood supply on the data. The dynamic data collection may be transformed into a smooth time-activity curve. However, the rate of substrate or ligand binding, or of its release, or of its degradation in the ROI, can only be assessed when the measurements differentiate between indicator and its labeled degradation products that recirculate into the ROI. Moreover, local blood perfusion, transport, and diffusion of the labeled indicator and/or its labeled degradation product need to be accounted for so as to arrive at meaningful data.

It is obvious that three principal challenges must be solved: one pertains to the choice of tracer and to its positioning on the indicator; the second involves the data acquisition in such a form that supply of indicator to the reaction site is quantified and, if needed, indicator and its labeled reaction product are registered separately. The third challenge entails application of an appropriate model for data analysis – one that corresponds as closely as possible to the true life-events in the ROI, and if needed, considers confounding factors including those from local perfusion, substrate transport, and diffusion.

Any attempt at quantitative measurement of a biochemical reaction demands acknowledgement of the following:

- Imaging or counting of a labeled substrate in the stationary or dynamic mode only registers the relative amount or rate of uptake and/or release of the radionuclide tracer, and not necessarily of the labeled substrate within the chosen ROI in the body.
- For the labeled substrate to serve as indicator, the radionuclide must remain bound to the labeled substrate during the time of observation at least until the wanted reaction occurs.
- Local blood supply influences the rate of transport of the indicator to, and tracer removal from, the site of substrate reaction. A diminished local perfusion, for example, may cause a reduced local uptake of the indicator and/or concomitantly release of tracer and may mimic a reduced reaction rate.
- Local transport and diffusion from the capillary system into the extracellular space and cells determine the rate of supply of labeled substrate to, and removal of tracer from, the site of substrate reaction and, thus, may significantly influence the measurement of a reaction rate.
- The indicator within the cell may escape reaction and be transported or diffuse back into the extracellular space and capillary vessels. This loss of tracer from the reaction site may mimic a reaction rate.
- The tracer alone may recirculate into the ROI where the reaction occurs. Depending on the relative amount of recirculating tracer, it must be separately measured for final data assessment.

To overcome these principal challenges of directly observing biochemical reactions within a selected ROI, the following are required (Feinendegen et al. 1981):

1. Knowledge of the type of biochemical reaction to be studied in terms of substrate/molecular target

interaction and biochemical fate of subsequent reaction products

2. Proper choice of radionuclide for labeling the substrate to be measured and for the type of imaging or counting instrument; for instance, a gamma-emitting radionuclide for conventional scintigraphic imaging (planar or tomographic mode), and a positron-emitting radionuclide for positron-emission-tomography (PET)
3. Optimal and usually stable positioning of the chosen radionuclide on the substrate in such a way that it becomes a useful indicator for tracing the wanted biochemical reaction
4. Measurement techniques (preferably in the dynamic mode) which generate time-activity curves for analyzing kinetics of labeled substrate, and, if needed, a second, separate indicator or tracer as internal standard
5. Data analysis with the help of models in compliance with local tissue physiology and biochemistry (compartment models usually); and
6. Proof of applicability of the method using collateral experiments

The above requirements 1, 2, and 3 are primary challenges for the expert in radiopharmaceutical chemistry. Requirements 4, 5, and 6 mainly address the nuclear medical specialist. Both specialists need to start by sharing attention to requirement 1. Usually more than one measurement is needed to describe the wanted reaction kinetics in vivo. Various applications of “dynamic multiple parameter analysis” have proven their effectiveness. The principal approaches will be briefly summarized in the sections below, and their modifications and applications will be described and discussed in various other chapters of this book.

1.5

Methods and Models for Measuring Biochemical Reactions

This section gives various methods and models with some examples that aim at observing biochemical reactions in vivo both in a given ROI by imaging mainly in the dynamic mode, and, secondly, by whole body observation through dynamic in vitro analyses of tracer radionuclides in exhaled breath, body fluids, and excreta, with or without concomitant measurements in the peripheral blood. Of course, the mea-

measurements must be quantitative, highly sensitive, and specific in order to relate to fine-tuned control mechanisms in the signaling networks of living tissue. Two principle avenues answer to these demands. One of them focuses on observing a chosen single compartment, for instance, in a region of interest (ROI), and the other concentrates on observing a labeled substrate in two or more compartments, which usually consist of circulating arterial blood and the one or more tissue compartments it supplies with substrate where the reaction takes place. In the single compartment approach, a dual-parameter analysis is nearly always indispensable. The observation of a labeled substrate in two or more compartments simultaneously already provides the multiple-parameter analysis, without which a biochemical reaction *in vivo* can hardly be assessed properly.

1.5.1

Observations of Labeled Substrate(s) in a Compartment (ROI)

1.5.1.1

Single Parameter Analysis:

Single Radionuclide on One Substrate

1.5.1.1.1

In Vivo Counting and ROI Imaging

In most instances, this approach does not allow one to measure a single biochemical reaction for reasons given above regarding the complicating factors. However, some measurements of tracer outflow from a given ROI may very well describe a reaction. This applies, for instance, to measuring lipid turnover in the myocardium using ^{11}C -palmitic acid or other suitably labeled fatty acids, provided that noise from recirculating non-specifically bound tracer in the ROI is minimal and that loss of indicator by simple back-transport/diffusion from the myocardial cells into the blood circulation has been excluded (Schelbert et al. 1986; Feinendegen 2000). Another example is the analysis of the effectiveness of membrane channel function in multi-drug resistance, for example, in breast cancer (see Chap. 27). The rate of washout of tracer from the cancer tissue here correlates directly with the channel function that is responsible for the degree of drug resistance. Because of easy pitfalls and misinterpretations, this mode of measurement re-

mains an exception yet an important one for imaging biochemical reactions.

1.5.1.1.2

In Vitro Counting Related to Regional or Whole Body Metabolism

In vitro counting of a tracer, for example in the patient's exhaled breath, at various times after administration of a labeled substrate may signal the reaction rate as it occurs at certain sites or anywhere in the body. A case in point is the study of intestinal absorption of labeled substrate such as ^{14}C -lactose given orally and measuring the appearance of ^{14}C - CO_2 in exhaled breath (Sasaki 1995). This serves to detect and define the common problem of "lactase deficiency" (see Chap. 14). Synthesis of hepatic proteins, e.g., albumin and/or fibrinogen, can be evaluated with carbon- or hydrogen-isotope-labeled amino acids in such conditions as cirrhosis or acute-phase response to inflammation (see Chap. 14). Total body changes in glucose utilization or production in relevant diseases, notably diabetes, have been studied with ^{14}C - or ^3H -labeled glucose under various conditions of glucose load with repetitive plasma and/or breath sampling for analysis of turnover, as discussed in Chap. 14. A relatively simple technique compares the amount of any ^{14}C -labeled precursor of glucose in gluconeogenesis that is converted to blood glucose against the amount converted to ^{14}C - CO_2 in breath and blood (De Meutter and Shreeve 1963). Such an approach might be adapted to clinical application for detection of early predisposition to diabetes mellitus. Many of such studies suggest increased glucose turnover in diabetes, which is consonant with other evidence using various tracers, such as ^{14}C , ^{13}C , ^3H , or ^2H , pointing to increased hepatic glucose production as a predominant effect of impaired insulin function. There is also evidence for an insulin effect on the genetic expression of key enzymes that switch intermediate 3-carbon substrates between oxidative and gluconeogenic fates (Taylor 1995).

With current techniques, some of these questions could perhaps be further explored *in vivo* with ^{11}C -glucose using PET, provided that the short half-life of ^{11}C allows for such observations. More futuristic is the possibility of analyzing the localization and biochemical transformation of administered ^{13}C -enriched glucose by MRI and MRS *in vivo* and/or *in vitro* over a certain period of time.

1.5.1.2

Dual Parameter Analysis:

Two Radionuclides in Different Positions on One Substrate

1.5.1.2.1

In Vivo Counting and ROI Imaging

A rather simple approach to overcome many of the complicating factors in the in vivo measurement of biochemical reactions in a given ROI uses a double-labeled substrate. This technique is applicable in case a biochemical reaction leads to cleavage of the substrate into two differently labeled products. Double labeling with two different tracers that only separate upon substrate cleavage has been used in animal and human studies. If one of the labeled products remains at the reaction site and the other rapidly leaves the site, this approach also allows one to observe the site of the reaction. Thus, the enzyme ribonuclease was double-labeled with ^{131}I and ^{51}Cr and used in mice and rats to determine in tissue sections the rate and site of accumulation and degradation of the enzyme in vivo (Schultze et al. 1964). Autoradiography showed that the enzyme had rapidly accumulated in the proximal tubules of the kidneys where it was degraded within an hour. This was indicated by the loss of radioiodine whereas the chromium remained on site by entering new bonds. Thus, the change in isotopic ratio in the proximal tubular cells indicated that the enzyme had been degraded at that site.

This method was chosen in the early 1970s to image the degradation of insulin directly in vivo at various sites in the human body (Feinendegen and Ritzl 1971; Ritzl et al. 1974). In preparing this measurement, the insulin was double labeled with ^{131}I and ^{51}Cr in such a way that it retained its biological function. Both radionuclides become free upon insulin degradation in vitro and in vivo. Animal studies showed the free ^{131}I to quickly leave the site of degradation via the circulating blood and to enter the iodine pool of the body, supplying iodine to the thyroid gland. However, ^{51}Cr entered new bonds at the site of insulin degradation and thus remained behind (Ritzl and Feinendegen 1971). The special advantage of this type of measurement is the fact that the two different tracers remain bound to the substrate on its way through circulating blood, its transport and/or diffusion, until the reaction occurs; the externally measured quotient of the two tracers on the substrate remains constant until substrate degradation. This

eliminates a number of complicating factors, as previously discussed. The rate of change of the tracer quotient upon insulin degradation is the signal to be measured in the observed ROI. In fact, the tracer leaving the reaction site is the crucial signal and the other one remaining on site serves as an internal standard. A correction for recirculating radioactive iodine, if needed, is relatively easy; it requires measurement of peripheral blood and/or of quotient changes in an ROI where insulin degradation does not occur.

Dynamic imaging of the whole body begins immediately after intravenous injection of the double-labeled insulin. The change in tracer quotient shows the insulin to be degraded almost exclusively in the liver of patients with normal glucose metabolism, giving halftimes of about 40 min. The rates of degradation in the liver of patients with various forms of diabetes mellitus has been found to differ substantially from one another and from that of non-diabetic individuals. In a limited study, juveniles with type I diabetes had a significantly enhanced rate of insulin degradation, with half times ranging around 20–30 min, whereas in a second type of non-insulin dependent diabetes with a high insulin level a grossly reduced degradation rate was recorded, with half times over one hour (Ritzl et al. 1974). Such measurements may help in differentiating types and pathogenesis of diabetes mellitus. Moreover, calculation of the rate of synthesis of circulating insulin may result from measured turnover data, as long as substrate concentration at a non-degrading site is constant at a given homeostatic equilibrium. This approach may also find application in quantitatively and locally assessing degradation and synthesis rate in vivo of other peptides and proteins.

A similar way of using two radionuclides on one substrate in different positions resulted in following the fate of isolated DNA for the first time in a living mammal (Friedrich et al. 1972; Meyers and Feinendegen 1975 a, 1975 b, 1976). DNA was simultaneously labeled with ^3H -thymidine and $5\text{-}^{125}\text{I}$ -2'-deoxyuridine. The latter is an analogue of thymidine but less readily incorporated into mammalian DNA in vivo and in many cells in vitro (Meyers and Feinendegen 1975). Following intravenous injection of the double-labeled DNA, the quotient of the two tracers is insensitive to local blood circulation, transport and/or diffusion. Upon DNA hydrolysis, both labeled pyrimidines are released and enter the re-incorporation pathway in DNA-synthesizing cells in the body. However, whereas

^3H -thymidine is readily accepted into DNA synthesis, $5\text{-}^{125}\text{I}$ -2'-deoxyuridine is discriminated against, so that after re-incorporation the quotient of the tracers in vivo changes in favor of ^3H -thymidine in tissue and the reverse holds for the quotient in the circulating blood. Consequently, the change in the tracer quotient in favor of ^3H -thymidine in any given tissue signals DNA hydrolysis. No change of quotient in the recipient cells, on the other hand, means that no hydrolysis has occurred and labeled DNA has been trapped as a whole (Feinendegen et al. 1973; Meyers and Feinendegen 1975, 1976). The data showed the probability of intercellular DNA transfer in lymph nodes in the examined mice.

In these studies, the labeled ^3H -thymidine served as internal standard and the disappearance of $5\text{-}^{125}\text{I}$ -2'-deoxyuridine against ^3H -thymidine in tissue signaled reutilization of thymidine in favor of its analogue following DNA hydrolysis to the nucleoside level. By using thymidine or a metabolically equivalent analogue labeled, for instance, with positron emitting ^{11}C or ^{18}F , together with $5\text{-}^{123}\text{I}$ -2'-deoxyuridine for DNA double labeling, comparative studies on the fate of injected DNA may succeed in humans.

The dual-tracer-on-one-substrate technique in conjunction with dynamic imaging leads to the following mathematical representation. With:

$C[A/B]T(t)$ = the quotient of radioactivity from the two tracers, with A representing the signal and B the internal standard in the tissue region T at time t , and

k = the rate constant of the reaction

$$dC[A/B]_T(t)/dt = -k \cdot C[A/B]_T(t)$$

and after integration

$$C[A/B]_T(t) = -k \cdot \int C[A/B]_T dt$$

The graphic display of this expression shows the slope, $-k$, to quantitatively describe the rate constant of the reaction in the observed tissue.

1.5.1.2.2

In Vitro Counting Related to Regional or Whole Body Metabolism

Following oral or intravascular administration of substrates labeled with two different tracers, for example ^{14}C and ^3H in two different positions of glucose, sev-

eral analyses are possible. After ingestion, the conversion of $1\text{-}^3\text{H}$ -glucose to $^3\text{H}\text{-H}_2\text{O}$ represents the rate of absorption and initial metabolism of glucose; this provides a control against which to measure the slower rate of $^{14}\text{C}\text{-CO}_2$ formation from $1\text{-}^{14}\text{C}$ -glucose; the rate difference indicates the extent of glucose diversion to organic products such as fat in obese subjects (Shreeve et al. 1971). Another example is the use of double-labeled (^2H and ^{18}O) water injected intravenously. The difference in rates of decline of ^2H in the peripheral blood signals H_2O turnover, whereas the decline of ^{18}O indicates H_2O turnover plus CO_2 production and excretion in body water. The difference in the rates of decline allows calculation of energy expenditure in the total body (Ritz and Coward 1995), as discussed in more detail in Chap. (12). An equivalent approach sequentially measures labeled catabolites of substrates labeled with one tracer in two different positions, for example, ^{14}C in position 1 or 6 of glucose (Kallie et al. 1968), or ^{14}C in position 1 or 2 of glycine (Nyhan 1984; see Chap. 14). In these examples, the ratio of rates of appearance of the labeled catabolite CO_2 signals the relative rates of particular metabolic pathways involving particular reactions and can sensitively detect pathological deviations.

1.5.1.3

Dual Parameter Analysis:

Two Different Labeled Substrates

1.5.1.3.1

In Vivo Counting and ROI Imaging

1.5.1.3.1.1

Subtraction Technique

In this category of methods, a labeled compound that as background noise disturbs the diagnostic signal is subtracted from the gross data in order to reveal the fate of the indicator in a biochemical reaction. A useful illustration of this approach gives the in vivo analysis of lipid metabolism in the myocardium with labeled fatty acids (Feinendegen 2000). The experience with double-labeled insulin, as referred to above, helped in the 1970s to develop techniques for measuring the metabolism of fatty acids in vivo in the myocardium (Freundlieb et al. 1978, 1980; Machulla et al. 1978; Feinendegen et al. 1981).

Fatty acids are essential sources of energy by way of beta-oxidation in the mitochondria of myocardium and muscle in general. Physiologically, fatty acids

may be stored in lipids for retrieval into energy-delivering metabolism on demand. In attempting to relate the measured data obtained by dynamic planar scintigraphy to the in vivo fate of labeled fatty acids in various segments of the myocardium, a major problem has been the exclusion of non-specific signals from the scintigraphic image.

Fatty acids are long hydrocarbon chains with a carboxyl group on one end and a methyl group on the other. They can be labeled in different positions. Thus, radioactive carbon may be placed within the carboxyl group and radioactive iodine such as ^{123}I may be placed along the hydrocarbon chain wherever there is a double bond between two successive carbon atoms; or ^{123}I can replace the terminal methyl group, for instance, on the 16 or 17 C-position. The latter thus becomes ω - ^{123}I -heptadecanoic acid. Labeled in various ways, fatty acids may retain the ability to at least partly participate in myocardial metabolism. No metabolic interference arises from labeling with radioactive carbon in the carboxyl group. When radioactive iodine replaces the terminal methyl-group, as in ω - ^{123}I -heptadecanoic acid, the rate of indicator transfer into the myocardial lipid pool is reduced compared to physiological fatty acid. Most of the accumulated indicator in the myocardium rather rapidly transfers to beta-oxidation with quick liberation of free iodine tracer into the peripheral circulation. After having become incorporated into the lipid pool, ω - ^{123}I -heptadecanoic acid has similar kinetics as the physiologically behaving carbon-labeled fatty acid and thus one can trace fatty acid transfer from the lipid pool into the beta-oxidation pathway (Feinendegen 1993). The advantage of terminally labeling with radioactive iodine is the use of conventional gamma cameras in planar or tomographic mode for analyzing in vivo fatty acid uptake and metabolism, especially the rate of lipid turnover. On the other hand, labeling with positron-emitting carbon requires the more expensive and labor-intensive PET, which allows observation of the unaltered fatty acid kinetics and quantification of substrate in tissue.

Depending on the type and labeling of fatty acid, only a certain fraction of the accumulating tracer becomes incorporated into the myocardial lipid pool (Feinendegen 1993, 2000). Degradation of fatty acids by beta-oxidation in the mitochondria is faster than the rate of fatty acid supply from the lipid pool for degradation. Upon full degradation, the free tracers, whether carbon or terminally bound iodine, quickly leave the degradation site via the circulating blood.

The rate of tracer disappearance from the ROI thus mirrors the rate of entrance of the indicator into the degradation pathway. However, contrary to the tracer carbon, the free iodine tracer rapidly reenters the ROI with the free iodine pool in the circulating blood. The catabolic iodine tracer thus adds to the signals from labeled fatty acid in the myocardium and obscures the fatty acid degradation signal in the dynamic mode of imaging.

The interfering signals from the pool of free iodine in blood and tissue have been separately counted following an intravenous injection of free radioactive iodine (Freundlieb et al. 1980; Feinendegen et al. 1981). The separate signals from the iodine pool were then used to compute the rate of release of the iodine tracer in the ROI as the signal of transfer of the labeled fatty acid from the lipid pool into rapid degradation in the mitochondria. In diseased heart muscle, tracer release may include fatty acid back diffusion or transport into the blood circulation. If needed, these two pathways can be separately observed, as described below in the discussion of the use of two labeled isomerically related substrates.

Thus with:

$C_{\text{TM}}(t)$ = radioactivity of labeled fatty acid in the myocardial lipids at time t , and
 k = rate constant of loss of tracer from the myocardium, indicating physiological transfer of indicator from the lipid pool into mitochondrial beta-oxidation, followed by rapid wash-out of free iodine tracer into the circulating blood

$$dC_{\text{TM}}(t)/dt = -k \cdot C_{\text{TM}}$$

and after integration

$$C_{\text{TM}}(t) = -k \cdot \int C_{\text{TM}} dt$$

As stated above, one method of correcting for signal noise from iodine tracer in the recirculation pathway requires a second injection of free radioactive iodine such as ^{123}I -NaI and its measurement at time of equilibrium distribution, t' , in tissue and blood. With

$I_{\text{(T)}}(t)$ = recirculating tracer after indicator degradation in tissue ROI at time t
 $I_{\text{(P)}}(t)$ = recirculating tracer after indicator degradation per volume of peripheral blood at time t
 $I_{\text{(T)}}^*(t')$ = injected standard tracer in tissue ROI at time t'

$$\begin{aligned}
 I_{(P)}^*(t') &= \text{injected standard tracer per volume} \\
 &\quad \text{of peripheral blood at time } t' \\
 C_{TM}(t) + I_{(T)}(t) &= \text{gross tracer in ROI image at time } t \\
 C_{TM}(t) &= [C_{TM}(t) + I_{(T)}(t)] - I_{(P)}(t) \cdot [I_{(T)}^*(t') / I_{(P)}^*(t')]
 \end{aligned}$$

Following subtraction of the metabolically released and recirculating tracer in the ROI, given by the expression $\{I_{(P)}(t) \cdot [I_{(T)}^*(t') / I_{(P)}^*(t')]\}$ from the total radioactivity in the ROI, $[C_{TM}(t) + I_{(T)}(t)]$, the net signals from the myocardial lipids, $C_{TM}(t)$, in the ROI were obtained. Other correction techniques based on graphical analyses of radioactivity-time curves have been developed and successfully applied when needed (van Eenige et al. 1987).

Besides ^{11}C -labeled fatty acid, such as $1\text{-}^{11}\text{C}$ -palmitic acid, or a fatty acid with radioactive iodine replacing the terminal methyl group, such as $\omega\text{-}^{123}\text{I}$ -heptadecanoic acid, various other terminally phenylated fatty acids and those with side chains, mainly labeled with radioactive iodine, are being used today in clinical cardiology (Machulla et al. 1980; Knapp and Kropp 1995; Feinendegen 2000). These derivatives have the advantage of minimal recirculation of free tracer after indicator degradation and thus obviate the need for correction procedures. All these labeled fatty acids allow the measurement of not only the esterification of labeled fatty acids into the myocardial lipid pool, but also to various degrees the relatively slow rate of lipid turnover with fatty acid transfer into beta-oxidation. These diagnostic investigations, at times in conjunction with imaging of local blood perfusion, have added to understanding the pathology of ischemic heart disease and have helped in the non-invasive diagnosis of non-ischemic cardiomyopathies (Feinendegen 2000). Other applications of labeled fatty acids for metabolic studies in the liver are discussed below (Hoeck et al. 1986).

1.5.1.3.1.2

Internal Standard Technique

This particular method uses two differently labeled compounds, one the initial substrate that enters the reaction chain of interest, and the other a substrate downstream in that chain (see Chap. 16). Both labeled substrates are pulse-injected into the circulating blood and have similar kinetics en route to the reaction site. Dynamic imaging at a given ROI begins soon after injection of the indicators. Provided the labeled reaction products or liberated tracers rapidly

leave the reaction site without recirculation, the release rates of one tracer gives the rate of the upstream reaction and the other gives the rate of the downstream reaction in the chain. The release rate of tracer from the downstream substrate thus serves as an internal standard against which the release rate of tracer from the upstream substrate is scaled.

This technique has shown its success in linking some forms of non-ischemic cardiomyopathies to a genetically determined deficiency of one of the acyl-CoA-dehydrogenases that catalyze beta-oxidation of short-, medium-, and long-chain fatty acids. A deficiency in one of these enzymes may be accompanied by no clinical symptoms at all; or it may cause more or less grave cardiac insufficiency, differing in childhood and adolescence. In order to test for the type of the enzyme failure in fatty acid degradation, the release rates of tracer from the myocardium following intravenous injection of ^{11}C -labeled fatty acids of different chain length were measured. The downstream substrate was ^{11}C -acetate, the final substrate in the degradation chain, serving as an indicator of oxidative metabolism and, thus, as an internal standard (Brown et al. 1987; Armbrecht et al. 1990). Its intravenous injection shortly preceded or followed that of each one of the different ^{11}C -labeled fatty acids. The difference between the tracer release rate from the upstream fatty acid of a given chain length and the downstream internal standard release rate indicated the severity of inhibition of fatty acid degradation due to the deficiency of the appropriate acyl-CoA-dehydrogenase (see Chap. 16). To be diagnostically valid, the examination demands a steady state myocardial metabolism, i.e., exclusion of effects from substrate concentration in the circulating blood, hormones, workload, ischemia, and tracer back diffusion. This type of internal standard application is an excellent example of detecting in vivo the localized effect of a genetically caused disorder, with a relatively high sensitivity and without substantial risk to the patient (Kelly et al. 1993).

1.5.1.3.2

In Vitro Counting Related to Regional or Whole Body Metabolism

Dual-indicator techniques are potent also for in vitro measurements. For instance, a ^{14}C -labeled substrate proximal to an enzyme in a reaction chain and a ^{13}C -labeled substrate distal to it, are simultaneously administered, and the rates of formation of the differently

labeled CO_2 are compared with each other. In this set, the second indicator serves as an internal standard, provided that the kinetics of both indicators is similar until they reach the reaction site. For instance, a crucial set of reactions of intermediary metabolism, concerning anabolic vs. catabolic fates of pyruvate, could be tested by simultaneously employing pyruvate and acetate, each carrying a different tracer such as ^{14}C and ^{13}C for measuring the rates of appearance of the differently labeled CO_2 in the exhaled breath. In this metabolic area, interpretations of altered ratios of formation of labeled CO_2 can be multiple and reaction identification is aided by analysis of tracers in relevant organic products such as glucose or ketone bodies. Possible applications could be in states of diabetes or excessive growth hormone (Shreeve et al. 1970). The breath CO_2 test for "lactase deficiency" using labeled lactose (see above) would be sharpened for better focus on intestinal lactase activity per se if both ^{14}C and ^{13}C should be used for dual labeling and simultaneous administration of labeled lactose and the downstream labeled product, glucose (see Chap. 14). In this case, there should be assurance of normal metabolism of the internal standard, glucose, e.g., no diabetes or obesity.

Regarding macromolecular synthesis, for instance of hepatic lipoprotein, the simultaneous use of ^{125}I -apo-A and ^{13}C -phenylalanine as a standard for general protein synthesis allows the quantification of the contribution to lipoprotein (LP) from the "good" apo-A (high-density LP, HDL) vs. general LP synthesis (Rader et al. 1993), as further discussed in Chap. 14. The kinetics of intestinal absorption of elemental calcium may be observed without contribution to data from subsequent post-absorptive metabolic fate, by the simultaneous use of two calcium isotopes, either of radioactive ^{45}Ca and ^{47}Ca (DeGrazia et al. 1965), or of stable ^{43}Ca and ^{46}Ca (Smith et al. 1996) (discussed in Chaps. 12 and 14). In this test, one of the two paired tracers is given orally and the other intravenously, with measurement of tracer quotients in urine or saliva. Such data has led to recognition of a genetic predisposition to impaired absorption of Ca^{++} as a prelude to osteoporosis. The kinetics of iron absorption can similarly be evaluated by the use of paired iron isotopes, be they radioactive or stable, one given orally, the other intravenously; subsequent measurements of tracer quotients in blood plasma and red cells may reveal, for example, the degree of Fe^{++} absorption in pregnant women (O'Brien et al. 1999) (see Chap. 14).

A more elaborate example of the double-labeling approach for in vitro counting is the study with long-term intravenous infusion of $[3\text{-}^{14}\text{C}]\text{-lactate}$ together with ^{13}C -bicarbonate and oral administration of phenylacetate at various intervals over a period of six hours, in order to estimate Krebs cycle activity and hepatic gluconeogenesis (Landau et al. 1995). During indicator infusion, the ^{14}C and ^{13}C activities have been analyzed in breath CO_2 , in blood glucose, and in urinary urea and glutamate from the excreted phenylacetylglutamine. This carefully designed investigation showed that in fasted normal subjects 80% of glucose production was via gluconeogenesis, whereas in fasting diabetic patients the metabolic state was more heterogeneous, gluconeogenesis being reduced to an average of about 45%. This lower contribution of gluconeogenesis to glucose production in fasting diabetic patients was explained as a consequence of remaining glycogen stores during the period of insulin withdrawal.

1.5.1.4

Dual Parameter Analysis:

Two Labeled Isomerically Related Substrates

Potentially powerful is the use of two isomeric substrates that are labeled by different tracers for simultaneous imaging, or with the same tracer for sequential imaging. Provided the two isomeric indicators share kinetics before reaching the site of the wanted reaction, measuring the two different rates of tracer accumulation or release at the chosen ROI gives the difference in the reaction rates of the two isomers.

The development and testing of two labeled isomerically related substrates, i.e., 15-(para-iodo-phenyl)-pentadecanoic acid (pPPA) and 15-(ortho-iodo-phenyl)-pentadecanoic acid (oPPA), stable-labeled with radioactive iodine (Machulla et al. 1980; Shreeve et al. 1984; Beckurts et al. 1985; Kaiser et al. 1990) provides an example of how one can observe the biochemical nature of the active site of an enzyme in vivo, while also exploiting this information for diagnostic purposes. The profound difference in metabolism of the para- vs. the ortho-iodinated species of phenylated fatty acid in humans suggests the involvement of a protein-binding site akin to that of enzymes such as tyrosine kinases and tyrosine phosphatases, which are widely involved in signal transduction, which is specially discussed in Chap. 4. The further curious differences in the vulnerabilities of reactions with pPPA and oPPA in hepatic lipid metabolism to noxious influences, such as chronic alcohol

exposure (Shreeve et al. 1984) or adriamycin (Feinendegen et al. 1996), emphasize the value of employing other pairs of labeled isomeric substrates in parallel in order to reveal the possible occurrence or mechanisms of, or susceptibility to, disease. Genetically related changes in enzyme-substrate interactions should be observable by this technique. Thus far, myocardial and liver metabolism has been investigated by these two fatty acid isomers and may usefully serve to exemplify the paired isomers method.

1.5.1.4.1

Myocardium

In the context of labeling fatty acids with radioactive iodine for studies on myocardial lipid metabolism, the 15-(para-iodo-phenyl)-pentadecanoic acid, pPPA, proved metabolically very similar to natural fatty acids; moreover, its catabolites are rapidly released from the reaction site and excreted. This eliminates the need for time-consuming signal correction. Today pPPA is widely used in diagnostic cardiology with appropriate imaging devices (Feinendegen 2000).

Upon labeling phenyl-pentadecanoic acid with radioactive iodine, two thirds of the iodine binds in the para position, yielding pPPA and one third in the ortho position giving the isomeric 15-(ortho-iodo-phenyl)-pentadecanoic acid, oPPA (Machulla et al. 1980). This oPPA was found in studies with rodents to share with pPPA practically identical kinetics in blood circulation, transport and diffusion (Beckurts et al. 1985). Both isomers entered into the cells in parallel. However, both were significantly less readily bonded in vitro to CoA by the enzyme acyl-CoA-SH-thiolase than were palmitic acid and ω -iodo-heptadecanoic acid, probably by steric hindrance of the phenyl group in the ω position. Thus, after 30 min incubation the binding was about 30% for oPPA and about 45% for pPPA, but about 80% for ω -iodo-heptadecanoic acid, and about 90% for palmitic acid (Kaiser et al. 1990).

Greater differences between the two isomers were seen in rodents regarding esterification into complex lipids in the myocardium. Whereas pPPA readily entered the lipid pool, oPPA did so only to a minor degree (Beckurts et al. 1985). Moreover, in contrast to pPPA, oPPA in vivo hardly crossed into the mitochondria for beta oxidation, but left the cells nondegraded by back diffusion or transport into the circulating blood. When applied to humans in an initial attempt to label the free fatty acid pool as an internal standard in the measurement of myocardial lipid turn-

over, oPPA contrary to expectation was observed to have a much lower rate of release, with half times ranging to several hours (Antar et al. 1986). Indeed, only traces of labeled catabolites of oPPA appeared in the peripheral blood and urine. That the myocardial trapping of oPPA occurred outside mitochondria in the human myocardial cells was experimentally confirmed using a double labeling approach at diagnostic coronary angiography in patients. Both tracers, ^{123}I from the phenyl group of the oPPA and ^{14}C from the carboxyl group were retained in parallel and there was no change in the isotopic ratio in the coronary sinus. Loss of oPPA from the myocardium appeared mainly due to back diffusion or transport (Kaiser et al. 1990). Thus, after a practically parallel rate of uptake of oPPA and pPPA into the cells, pPPA may serve as an indicator of fatty acid metabolic degradation, while oPPA almost exclusively signals the rate of back diffusion or transport. It therefore appeared reasonable to use these two isomers to distinguish between losses of tracer from the myocardium by beta oxidation, with pPPA, versus back diffusion/transport, with oPPA (Feinendegen 1993).

The dual tracer technique with oPPA and pPPA both labeled with ^{123}I was applied diagnostically in patients with dilated cardiomyopathy (Feinendegen et al. 1995). The intravenous pulse injection of oPPA preceded the injection of pPPA by 50 min. After another 50 min, ^{123}I -NaI was intravenously given to correct for labeled catabolites in the tissue ROI by image subtraction, as referred to above. Regional uptakes of the two indicators did not differ between patients and control subjects. However, the release rates differed from control in at least 66% of the patients and the scatter was larger inter-individually than intra-individually. Since the release rate of tracer from oPPA indicated mainly back diffusion/transport, and that of pPPA both beta oxidation and back diffusion/transport, the difference between the two release rates are assumed to mainly give the rate of beta oxidation in the observed myocardial region. In this manner, three different patterns of lipid turnover appeared in these patients: (1) predominantly increased beta oxidation; (2) predominantly decreased beta oxidation, in part with increased back diffusion/transport; and (3) predominantly increased back diffusion/transport. These significantly distinct patterns of myocardial lipid turnover in different patients with non-ischemic cardiomyopathies express different pathologies at the molecular level and may involve genetically determined predispositions.

1.5.1.4.2 Liver

Following studies of in vivo liver metabolism with ω - ^{123}I -heptadecanoic acid (Hoeck et al. 1986), the paired-isomers method with labeled fatty acids appeared even more revealing. As was shown for the myocardium, pPPA and oPPA in normal rats also had almost identical kinetics in the circulating blood before reaching the liver tissue cells. Both then had distinct patterns of esterification into the various lipid fractions, and this was independent of local blood perfusion at the anatomic site (Feinendegen et al. 1996). Adriamycin, which stimulates hepatic lipid synthesis, caused a highly significant change in rats in the ratios of liver uptake of pPPA and oPPA as into various lipid fractions compared to normal liver. The quotient oPPA/pPPA was 2.6 for whole liver in normal rats, and significantly lower at 1.5 after adriamycin treatment. The corresponding quotients oPPA/pPPA in liver triglycerides were significantly different by a factor of 5, with 2.0 in normal rats and 0.4 after adriamycin treatment (Feinendegen et al. 1996). By contrast, in mice exposed chronically to ethanol there was increased conversion of oPPA to liver triglycerides (TG) relative to nonexposed mice, with no effect of ethanol on incorporation of pPPA. In addition phasic differences occurred in the ethanolic mice in decline of activity in TG, as in total liver, from oPPA as well as from ω - ^{123}I -heptadecanoic acid or ^{14}C -stearic acid, but not from ^{14}C -oleic or palmitic acids (Shreeve et al. 1984). The quotient change for paired fatty acid precursors converted to circulating triglycerides may be a sensitive diagnostic indicator of pathological hepatic lipid metabolism. Further study is also warranted in the context of genetically determined alterations in reaction rates of hepatic lipid synthesis.

In a preliminary clinical application, trace amounts of ^{123}I -labeled pPPA and oPPA were given intravenously one shortly after the other. The sequences of scintigrams displayed each indicator separately and as a quotient of the two. Indeed, the significant change of the normal pPPA/oPPA quotients in liver disease appeared as a clinically useful indicator of a disturbance of lipid metabolism (Ebert et al. 1993). Previously, local pathological alterations in liver metabolism could only be detected by microscopic examination of tissue specimens.

1.5.1.5 Dual Parameter Analysis: Two-Isotope Effect on Labeled Substrate

For in vivo analyzing the function of substrate/ligand binding to its enzymes/receptor, the use of the isotopic effect of deuterium (^2H) in place of hydrogen (^1H) in hydrogen bonding to carbon was introduced by Fowler in a seminal study (Fowler et al. 1988; see Chap. 20). When ^2H replaces ^1H , where a carbon-hydrogen bond normally forms during substrate interaction with its specific target, the ensuing rate of binding is altered. The reason for this is the increased stability of a $\text{C}-^2\text{H}$ bond compared with a $\text{C}-^1\text{H}$ bond. By choosing this dual parameter technique for in vivo imaging of an appropriately labeled substrate, most confounding factors associated with in vivo imaging of biochemical reactions, as outlined above, can be excluded. If the concentration of receptor on site is to be assessed by the indicator ligand, the increased bond stability is an advantage in that it reduces the effect of an unfavorable relationship between a relatively low receptor concentration and a high rate of labeled substrate binding and loss.

This approach was employed for studying the function of monoamine oxidase, MAO, in the brain of humans and lower mammals. As given in more detail in Chap. 20, MAO type B binds L-deprenyl covalently and irreversibly to a co-factor that arises during MAO-B-catalyzed oxidation. In this way, L-deprenyl is a suicide inactivator of the enzyme. L-deprenyl labeled with ^{11}C therefore represents a potent indicator for measuring site and function of MAO-B at a given ROI in the brain by PET.

The hydrogen at various sites of ^{11}C - or ^{14}C -labeled L-deprenyl was replaced with deuterium. Deuterated and non-deuterated radioactively labeled L-deprenyl were then intravenously administered in experiments with baboons and mice and later in human studies. In the latter, PET imaging showed the two L-deprenyls to accumulate mostly in the basal ganglia and the thalamus, with lesser intensities found in the frontal cortex and the cingulate gyrus. Binding was lowest in the parietal and temporal cortices and the cerebellum. When the deuterium was incorporated in the methylene group of the propargyl group of L-deprenyl, an isotope effect appeared in that the rate of tracer uptake and binding was significantly reduced, as was the rate of its release compared to the control. The data thus established in vivo that the alpha-carbon-hydrogen bond on the propargyl group is a sin-

gular or major rate-limiting step in oxidation by MAO-B.

Obviously, this particular *in vivo* method appears powerful for investigating on the one hand the specific site with which a substrate/ligand binds to its specific enzyme/receptor, and on the other for observing the consequences of structural alterations on the binding due, for instance, to the action of molecular signaling, or to genetic polymorphism in the pathogenesis of disease. Moreover, this type of information may help to structurally tailor a substrate for developing drugs for effectively altering biochemical reactions *in vivo*.

1.5.2

Parallel Observation of Labeled Substrate in Two or More Compartments

Measuring the transfer of a given indicator between physiological compartments, such as between blood and tissue, body and urine, body water and tissue, etc., compartmental analyses have found wide application in nuclear medicine since its early days (see Chaps. 7, 8). Presently, with tracers and labeled indicators being available for imaging biochemical reactions, compartmental analyses are routinely used to assess a biochemical reaction by the kinetics of indicator transfer to, or loss from, or both, the site of a reaction – for instance within a chosen ROI. Two situations arise, one in which the indicator is practically irreversibly bound over the period of observation, and the other where the indicator after accumulation in the observed ROI leaves the site into the circulating blood or is degraded with release of tracer. The accuracy of compartmental analysis increasingly suffers with the number of interrelated compartments and complexity of the model. The observation of indicator binding alone, or together with subsequent indicator dissociation from its target site, in a chosen ROI makes the one- and two-tissue compartment analysis a near optimal tool. Chapters 7 and 8 extensively discuss modeling and tracer kinetics. This section will briefly introduce the compartmental analysis of reversible and irreversible indicator accumulation.

Usually, a single labeled substrate suffices for compartmental analysis of reversible or irreversible accumulation of indicator. The compartment model includes indicator supply from arterial blood to the tissue compartment where the reaction is to occur. The corresponding rate is expressed as clearance of indicator from the arterial blood plasma, and the rate of in-

indicator return into the blood is expressed by a rate constant. In this way, a dual parameter analysis results. A one-tissue compartment may suffice for describing reversible accumulation or indicator binding in tissue. A two-tissue compartment is composed of the extravascular tissue space of free indicator, i.e., the substrate pool in tissue, from where the indicator enters the irreversible bond to its target, which is denoted as the second tissue compartment. In either case, if the density of a defined receptor in terms of receptor concentration at the target site is the goal of measurement, the amount of indicator available and its binding affinity to the target are crucially important.

Obviously, the indicator must be structurally compatible with its specific target molecule, be it receptor, transporter, or enzyme in the interstitial space, and on or within a cell. In order to take advantage of the indicator's irreversible binding to its target, and thus its accumulation in the ROI as a function of the reaction rate, appropriate molecular tailoring of the indicator is a powerful approach, as discussed below in the context of glucose metabolism.

The actual measurement begins with the intravenous injection of the indicator and requires frequent scintigraphic recording of the quantities of the indicator in the ROI under study, and parallel analyses of indicator concentrations in samples of the arterial blood plasma, from where the indicator is supplied to the reaction site. The assumption, of course, is the rapid transfer of indicator from the blood to this site. These measurements result in a set of two time-radioactivity curves, one giving the temporal changes of indicator concentrations within the arterial blood plasma, and the other gives the corresponding changes of radioactivity within the ROI.

The analysis of the two curves describing the indicator concentration within arterial blood plasma and the ROI radioactivity may furnish the desired information regarding the biochemical reaction to be studied, as explained below. Nevertheless, various caveats need attention regarding the complex kinetics of indicator supply to the reaction site, the reaction itself and, if applicable, transfer of labeled reaction product away from the reaction site in the ROI with possible recirculation. The mathematical treatment of the curves may favor computerized fitting procedures with input of the model-given variables. Another choice is the graphic display of reaction rates and rate constants. In this section, the graphic analyses are briefly summarized and presented with examples of application. More details are in Chap. 7.

1.5.2.1

Labeled Substrate in One-Tissue Compartment Model

The one-tissue compartment model describes the indicator's arrival and accumulation at the reaction site via the circulating blood without having passed through a functionally relevant second compartment. The model also takes into consideration the release of indicator from its site of accumulation and removal by the circulating blood. This approach applies, for instance, to studying reversible transport across a biological barrier within a given ROI. Thus, with

$C_T(t)$ = radioactivity in extravascular tissue space at time t ,

$C_P(t)$ = radioactivity per volume blood plasma at time t ,

K_1 = clearance of indicator from blood plasma to the extravascular tissue space (radioactivity/min \times volume)

k_2 = rate constant of indicator release from the extravascular tissue space to blood, (radioactivity/min \times mass)

$$dC_T(t)/dt = K_1 C_P(t) - k_2 C_T(t) \quad (1.11)$$

After equilibrium is reached between indicator concentration in blood and tissue,

$$K_1 C_P(t_{\text{equ}}) = k_2 C_T(t_{\text{equ}})$$

and the two measured time-radioactivity curves in blood plasma and tissue run in parallel. The ratio of the two measured values $C_T(t_{\text{equ}})/C_P(t_{\text{equ}})$ is equal to k_1/k_2 and expresses the tissue mass into which distributes an amount of indicator cleared per unit volume of blood plasma. This value is conventionally called the distribution volume.

By integration of Eq. 1.11,

$$C_T(t) = K_1 \int C_P dt - k_2 \int C_T dt \quad (1.12)$$

This equation may be rearranged in various ways leading to linearized expressions. One (Gjedde and Diemer 1983) is:

$$C_T(t)/\int C_P dt = K_1 - k_2 \int C_T dt / \int C_P dt \quad (1.13)$$

The graphical display of this equation results in a linear plot that extrapolates to the intercept at the value of K_1 and has the slope of $-k_2$.

Another way of linearization of Eq. 1.12 is (Logan et al. 1990):

$$\int C_T dt / C_T(t) = K_1 / k_2 \int C_P dt / C_T(t) - 1 / k_2 \quad (1.14)$$

Here, the linear portion of the plot gives the intercept as $-1/k_2$ and the slope describes the distribution volume K_1/k_2 .

The analysis thus results in the values of the clearance and the rate constant that governs back transport of the indicator from the tissue into the circulating blood. If such measurements are made at different substrate concentrations, which the indicator traces, the resulting rate constants may be interpreted in terms of first order reaction kinetics expressed by the Michaelis-Menten equation, described above.

1.5.2.1.1

Active Transport of Substrate

The one-tissue compartment model serves to quantify reversible transport of substrates across biological barriers such as the blood brain barrier (BBB). In analyzing glucose transport across the BBB at various blood glucose concentrations, ^{11}C labeled 3-O-methylglucose was employed as an indicator that is transported across the blood brain barrier similarly to d-glucose but is not metabolized (Feinendegen et al. 2001). It enters the free glucose pool in brain tissue and returns from there into the circulating blood. The potential of a very small second tissue compartment is neglected here. In the absence of a second tissue compartment, the indicator accumulation in the brain with time after indicator injection reaches equilibrium with the indicator concentration in blood plasma and the two time-radioactivity curves run in parallel. This indicates the reversible transport of this glucose analogue across the BBB between the capillary circulation and the extravascular free glucose pool in tissue. Diagnostic studies in humans showed that the rate constant k_2 indicating indicator outflow from the extravascular tissue space to blood differed among individuals to a considerably greater extent than the clearance rate of inflow, K_1 . Measuring these values throughout the cerebral cortex at different plasma glucose concentrations revealed a highly significant inter-individual but not intra-individual variability more for k_2 than K_1 at normal and elevated blood glucose levels. The results were analyzed in the context of present day knowledge of glial cell biochemistry and indicate highly individualized glial cell function, possibly genetically determined (Feinende-

gen et al. 2001). Indeed, the data suggest the existence of individually regulated control mechanisms of glucose metabolism in the brain.

1.5.2.2

Labeled Substrate

in Two-Tissue Compartment Model

The two-tissue compartment model contains, for instance, an extravascular tissue space into which the indicator enters from the circulating blood and from where it leaves, either by back transfer into the circulating blood or by irreversible binding to its target at the tissue site under observation; this irreversible binding site presents the second tissue compartment. The resulting two time-radioactivity curves, one from arterial blood plasma and the other from the tissue ROI, tend to converge, i.e., the tissue ROI curve rises where the one from blood plasma falls. Again, curve-fitting procedures as well as graphic displays are common for the determination of rate constants. Analogous to the one-tissue compartment analysis, here again the graphic approach is chosen to show the rate constants of indicator transfers in the ROI (Patlak et al. 1983; see Chap. 7). Thus, in a simplified manner with:

- $C_T(t)$ =radioactivity in extravascular tissue space at time t
- $C_T(t) = C_{TF}(t) + C_{TM}(t)$
- $C_{TF}(t)$ =radioactivity in the extravascular pool of free indicator in tissue at time t (compart. 1)
- $C_{TM}(t)$ =radioactivity irreversibly bound in extravascular tissue space at time t (compart. 2)
- $C_P(t)$ =radioactivity per volume blood plasma at time t
- K_1 =clearance of indicator from blood to extravascular pool of free indicator in tissue (radioactivity/min \times volume)
- k_2 =rate constant of indicator release from extravascular free indicator pool to blood (radioactivity/min \times mass)
- k_3 =rate constant of irreversible binding of radioactivity in extravascular tissue space (radioactivity/min \times mass)

the following differential equation arises for tissue compartment 1:

$$dC_{TF}(t)/dt = K_1 C_P(t) - k_2 C_{TF}(t) - k_3 C_{TF}(t) \quad (1.15)$$

and for tissue compartment 2:

$$dC_{TM}(t)/dt = k_3 C_{TF}(t) \quad (1.16)$$

Following integration of Eq. 1.16, tissue compartment 2 becomes:

$$C_{TM}(t) = k_3 \int C_{TF} dt \quad (1.17)$$

Also, at equilibrium between $C_P(t)$ and $C_{TF}(t)$:

$$K_1 C_P(t) = k_2 C_{TF}(t) + k_3 C_{TF}(t) \quad (1.18)$$

and by rearranging Eq. 1.18 tissue compartment 1 becomes:

$$C_{TF}(t) = \frac{K_1}{k_2 + k_3} \cdot C_P(t) \quad (1.19)$$

Because the measured $C_T(t)$ covers the 2 compartments $C_{TF}(t) + C_{TM}(t)$, Eqs. 1.19 And 1.17 need be added:

$$C_T(t) = \frac{K_1}{k_2 + k_3} \cdot C_P(t) + k_3 \int C_{TF} dt \quad (1.20)$$

and by substituting Eq. 1.19 into Eq. 1.20:

$$C_t(t) = \frac{K_1}{k_2 + k_3} \cdot C_P(t) + k_3 \cdot \frac{K_1}{k_2 + k_3} \int C_P dt \quad (1.21)$$

Dividing both sides of Eq. 1.21 by the measured $C_P(t)$:

$$\frac{C_T(t)}{C_P(t)} = \frac{K_1}{k_2 + k_3} + k_3 \cdot \frac{K_1}{k_2 + k_3} \frac{\int C_P dt}{C_P(t)} \quad (1.22)$$

Thus, the parallel measurements of $C_T(t)$ and $C_P(t)$ at given time intervals allow one to plot Eq. 1.22 graphically, and the straight line in the plot extrapolates to the intercept value $[K_1/(k_2 + k_3)]$, the slope given by $[k_3 K_1/(k_2 + k_3)]$.

Therefore, dividing the slope $[k_3 K_1/(k_2 + k_3)]$ by the intercept $[K_1/(k_2 + k_3)]$ yields k_3 , the rate constant of the irreversible binding.

1.5.2.2.1

Enzyme-Substrate Interaction

The two-tissue compartment model is most widely used for measuring glucose metabolism by labeled 2-deoxyglucose (Sokoloff et al. 1977). This glucose analogue is singularly suited to in vivo observation of the phosphorylation of d-glucose, the first step in glucose metabolism. D-glucose-phosphate is not further metabolized but is trapped at the reaction site. Thus, 2-deoxyglucose enters the free glucose

pool in tissue in a manner similar to that of glucose, for instance, via the BBB into glial cells, where it either binds to its target molecule hexokinase to become phosphorylated and trapped, or from where it returns to the circulating blood. By labeling deoxyglucose with ^{14}C a superb indicator was introduced for the experimental in vivo observation of the rate of glucose phosphorylation throughout the brain, by autoradiographically measuring the accumulation of the indicator in tissue slices from the entire brain. The analysis demonstrated that local tracer accumulation, with full consideration of local flow, transport, and difference in binding affinities between d-glucose and its analogue, correlated with the local rate of d-glucose phosphorylation (Sokoloff et al. 1977). This seminal work opened a new field of studies in the living brain, which relies predominantly on d-glucose for energy. A normal human brain consumes on average about 120 g of d-glucose per day.

Following extensive experimental work with ^{14}C -labeled 2-deoxyglucose, labeling with the positron-emitting ^{18}F introduced the ^{18}F -2-deoxyglucose (FDG) for in vivo imaging in humans with PET (Gallagher et al. 1977). For quantifying the rate of d-glucose phosphorylation in the human brain with the help of the two-tissue compartmental model, accumulation of FDG in various brain regions is dynamically imaged over a given period of time in parallel with measuring the concentration changes of indicator in arterial blood plasma. Other tissues such as myocardium and malignant tumors are similarly examined with FDG using the dual parameter approach. Indeed, FDG is presently by far the most widely used indicator for metabolic studies in vivo using PET (Phelps et al. 1979; Reivich et al. 1979; Delbeke et al. 2002). Various applications are presented in this book (see Chaps. 17, 18, 20, 27).

Regarding glucose metabolism in the human brain, one may summarize that activated neurons in the brain trigger an increased amount of glucose into metabolism mainly in the glial cells adjacent to neurons. This appears intimately connected with an increase in local cerebral blood perfusion. Changes in both local glucose consumption and blood perfusion allow one to localize neuronal stimulation in the living brain in specified motor, sensory, and mental activities. Thus, brain function mapping has become a reality. On the other hand, the state of total relaxation as in deep yoga meditation has been seen to cause the entire brain and not just certain regions to be affected, and different individuals reacted either by a decrease or an increase in global glucose metabolism

(Herzog et al. 1990/1991). In clinical practice, brain PET imaging with FDG is now a routine procedure in patients suffering from circulatory disorders including cerebral ischemia, tumor, and neural degeneration such as Alzheimer's disease. In current brain research and clinical medicine functional MRI of local blood perfusion increasingly substitutes for or complements the classical FDG studies on cerebral energy metabolism. Regarding effects of genetic determinants in controlling local cerebral energy metabolism, it appears tempting to correlate measurements of glucose metabolism or local blood perfusion with those of neurotransmitter and receptor activity in the same and interconnected brain regions.

In the myocardium, accumulation of FDG in relation to labeled fatty acids may indicate a shift toward anaerobic metabolism for energy supply in areas of diminished blood flow with reduced oxygen supply. Thus, tissue vitality in ischemic myocardial regions can be correlated with relatively increased levels of glycolysis. This test in conjunction with coronary blood flow measurements may also help to distinguish between stunned and hibernating myocardium and sometimes appears crucial in deciding, for instance, on coronary bypass surgery. Myocardial imaging with FDG in cardiological research and practice has been extensively reviewed (Schroeder and Schelbert 2000; see Chap. 17).

The demand for FDG with PET currently also rises in the diagnosis of malignant tumors (Delbeke et al. 2002). Because of preferential glucose consumption for energy supply in many cancerous tissues, whole body imaging with FDG can reveal the site and often also the type of a tumor and its metastases several months earlier than their appearance in conventional structural images with x-ray or MRI. Depending on tumor type, tumor glucose consumption is highly sensitive to chemical and radiation therapy. By its quick response, a reduction in FDG uptake in a tumor soon after treatment may be an early sign of long-term therapeutic efficiency and allows for individual planning for optimal therapy. Here, also attempts at gene therapy and gene expression in vivo, as discussed below and in Chap. 30, need be included in the potential treatment modalities.

1.5.2.2

Ligand-Receptor Interactions

Probably the most frequently applied nuclear medical procedure in current molecular imaging in vivo ad-

dresses ligand-receptor interactions. Both, the one-tissue and two-tissue compartment models are indispensable in the quantification of reaction kinetics.

The concept of specific receptors on cell membrane surfaces being sensitive to specific circulating substrates was first advanced in 1900 by Paul Ehrlich (Rensberger 1996). Now it is estimated that about 40% of the genome codes for such receptors. They are sensitive to a variety of chemical messengers such as hormones, growth factors, neurotransmitters, and pharmacological agents, which usually circulate in the blood and interstitial space. Most messengers between cells function as ligands through binding to receptors on membranes. These receptors typically extend through the cell membrane, having external domains for receptivity and internal domains for attachment to specific cell proteins involved in signal transmitters. Bonding of ligand triggers the receptor into phosphorylation or transphosphorylation in the region of the internal domain, as outlined in Chap. 4. Signal transductions through further protein-protein interactions inside the cell directly determine enzyme activities for metabolism or are transmitted to the nucleus, causing alterations in gene expression.

There is much evidence to indicate that polymorphisms of genes for receptors change the effectiveness of ligands and underlie various disease states. To cite an example: insulin resistance in Type II diabetes may be caused by heritable abnormalities of insulin receptors on the surface of peripheral target cells (Stern 2000; see Chap. 14). Another: genetically determined changes in serotonin receptor function in the mid-brain or even on platelets can be linked to predisposition to depression or alcoholism (see Chaps. 4, 14). Another: different phenotypes for estrogen receptor (ER) activity in breast carcinoma can be correlated to different patterns of gene expression (Gruvberger et al. 2001). And: mutations determining somatostatin receptor subtypes appear to account for differing responses of acromegalic patients to octreotide (Ballare et al. 2001). The challenge in nuclear medicine is to devise practical ways, by imaging or other means, to detect these gene-related abnormalities by differential binding of well-designed radioactively labeled ligands, which have physiological or pharmacological effects on the targeted receptors.

A particular advantage to nuclear medicine has been the appreciation that cell surface receptors are, in general, excessively or liberally expressed in tumor cells. One fruitful area has been the development of

radioactively labeled oligopeptides, which correspond to or mimic a natural ligand for some particular receptor (Boerman et al. 2000). Relatively well developed is the use of labeled variants of an octreotide, an analog of the peptide hormone somatostatin. This hormone mainly functions by inhibiting the secretion of other hormones. The ^{111}In -labeled-DTPA-octreotide is successfully used for imaging of neuro-endocrine and gastro-entero-pancreatic tumors, breast carcinoma, and lymphoma. Besides their advantageous diagnostic use, octreotides are in therapy trials, for instance in the form of ^{111}In -labeled DTPA-octreotide and ^{90}Y -labeled DOTA-Tyr3-octreotide. This use demands internalization of the receptor-ligand complex and long-term retention. Other peptides that have potential for imaging receptors on tumor cells include vasoactive intestinal peptides such as cholecystokinin, bombesin, and calcitonin (Boerman et al. 2000). Among hormones or analogues that have been labeled and found useful for tumor imaging are ^{18}F -labeled fluoro-estradiol, for instance for estrogen receptor-carrying breast tumors (Silverman et al. 1998) and ^{131}I - or ^{123}I -labeled meta-iodo-benzylguanidine (MIBG) for neural crest tumors (Shapiro et al. 1995). Still another class of compounds for tumor receptor imaging encompasses pharmaceutical agents, e.g., ^{18}F - or ^{131}I -labeled tamoxifen analogues for estrogen receptor-carrying breast tumors (Silverman et al. 1998). Similarly, angiogenesis (and apoptosis, too) are now amenable to ligand-receptor imaging. Indeed, targeted tumor-receptor imaging in the broadest sense is currently one of the most rapidly evolving nuclear medical techniques in clinical oncology (Kim and Yang 2001).

The special case of neuroreceptor stimulation by neurotransmitters at neuronal synapses results in conversion to an electrical signal. Neurotransmission in the living brain is being extensively investigated with some clinically important results for diagnosis and therapy planning, as discussed later in this book (see Chap. 18). Labeled neurotransmitters are crucial in functional brain mapping in vivo regarding motor, sensory, or pure mental activity. Following initial animal studies (Firnau et al. 1976), neurotransmitter imaging in the human brain began with the dopamine receptors (Wagner et al. 1983). Many such investigations now also pertain to measuring receptor availability and, dependent on post-synaptic receptor activity, to on-site concentration of neurotransmitters such as dopamine, serotonin, acetylcholine, in neurological and psychiatric disease or intoxication states

(Laruelle 2000), as discussed in more detail in Chaps. 18, 19. Useful information has accumulated with radioactive analogues of neurotransmitters, which behave according to the “occupancy model” of straightforward competition between natural ligand and labeled analogues. However, some of the labeled analogues do not follow the degree or kinetics of binding expected from the “occupancy model,” which has led attention to effects of the cycling of receptors between internalization and externalization and perhaps of other factors which may determine substrate binding. Such investigations complement the data obtained by FDG imaging of regional cerebral glucose metabolism or by corresponding functional MRI of local cerebral blood flow, as well as by intracerebral magneto-encephalography (MEG). Indeed, these various approaches in unison today open synoptically different kinds of views on the living brain and occupy a major research field of applied neurobiology, with revolutionizing consequences regarding understanding of neural and mental function.

Promising radioactively labeled ligands for receptors on leukocytes, monocytes, and lymphocytes are under investigation for imaging infection/inflammation, and on thrombi for detecting deep vein thrombosis and pulmonary emboli (Boerman et al. 2000; see Chaps. 10, 11). Beta-adrenergic receptor density in the myocardium in heart failure, ischemic heart disease, non-ischemic cardiomyopathy, and after heart transplantation has been evaluated with ^{123}I -labeled MIBG and other tracers (Syrota et al. 1995; Bengel et al. 2002).

Receptor imaging is likely to be extended to all body tissues and many diseases and be directed to questions regarding receptor sites, density, function, and regulatory susceptibility. As suggested by the multiplicity of involved genes and/or gene mutations in determining for instance estrogen receptor activity in breast cancer (Gruvberger et al. 2001) and insulin receptor activity in insulin resistance (Stern 2000), genetically determined nuances of individual responses to labeled ligands can be expected for various other kinds of receptors as well. Other possible modifications of receptor activity come from “epigenetic” reactions, such as acetylation, phosphorylation, or methylation of amino acids in nuclear histones, for example, in the context of the new “histone code” hypothesis (Maher 2001). Moreover, symbiotic effects on a specified target from exposure to differing levels of various natural ligands circulating in the blood need consideration.

Present day advances in this type of imaging are rapid and diversified and will undoubtedly open many new biochemical and cell biological systems to in vivo observation with reference to genetic control.

1.6

Probing for Gene Expression In Vivo

Over the past decade, in vivo gene expression has become a major research area with profound implications to clinical diagnosis and therapy, especially in oncology. The analytical techniques largely rely on the two-tissue compartment model. With the decoding of the human genome, new impetus is given to this field, which like hardly any other nuclear medical development relies on the intimate interdisciplinary interaction between cell and molecular biology and non-invasive in vivo imaging procedures, including radiopharmaceutical work.

Gene expression in cells and tissues may be measured by the activity of gene products, as referred to in various sections above, for instance, in the context of ligand-receptor imaging. In addition, the expression and efficiency of groups of correlated genes may become observable by combining various techniques discussed above for in vivo measurements of circuits of biochemical reactions dependent on these genes. The effect of a signaling pathway on one or more biochemical reactions within a functional circuit may thus eventually be unraveled in vivo and correlated with gene expression.

A direct way to analyze gene expression in vivo employs radioactively labeled probes that may bind to genes or their transcribed RNA. Such labeled markers need complementary binding to the DNA or RNA nucleotide chain, and thus consist of chains of a limited number of nucleotides. Such relatively small single-chain oligonucleotides serve as “antisense” markers defining precise nucleotide sequences. Initial work with such radioactively labeled antisense single oligonucleotides in vitro and animal experiments show the feasibility of the approach for diagnosis and therapy, as extensively presented in Chaps. 28, 30. A serious limitation is the relative instability of oligonucleotides through the action of nucleases; as a consequence, one tries to increase oligonucleotide resistance against nucleases by attaching metabolically blocking end groups. Another limitation lies in the intracellular barriers and the relatively small numbers

of DNA or RNA targets in cells. This causes rather weak signals for imaging unless the target number is amplified, which is still an unsolved problem. The situation is different for enzyme-catalyzed reactions where repetitive binding of labeled substrate as indicator allows a relatively large image signal. Other caveats arise through potentially interfering background radioactivity in whole system images after administration of labeled antisense probes. More confounding factors relate to the fate of large molecular indicators on the way through interstitial spaces to reaction sites, general complications of in vivo measurement of biochemical reaction, as referred to previously. One may expect that these problems will eventually be solved.

The other approach focuses on measuring specific gene transcription products, be they enzymes or receptors, as outlined above. This way is especially effective for assaying the success of gene transfer into cells in the course of attempts at gene-based therapy, be it for tumor cell killing or for altering cellular control or protective systems. Here, the primary concern is to localize the transferred gene and to describe its expression. When the transferred gene is not easily accessible to being observed by its transcription product, the goal is to attach a second gene whose transcription product can report the activity of the primary gene when both genes have the same promoter. Chapter 30 extensively discusses the progress in this field.

In vivo observation of gene expression may limit its focus to a single gene that has been stably transferred and is resident in the genome in a cell system. Or such observation may reveal the need for the *ad hoc* transfer of a gene as a desired therapy for eradicating a tumor or changing cellular function. The latter scenario is in early development, particularly challenging and demanding attention as to selection of genes for transfer, to the construction of gene groups including the reporter gene, to the techniques of transfer, and to the choice of the optimal indicator for analyzing the location and activity of the transferred gene or its reporter gene.

One example should suffice here to illustrate the potency of gene transfer for both tumor therapy and monitoring of gene expression by in vivo imaging of a reporter gene. The most widely used gene in the context of developing gene transfer and in vivo imaging of gene expression codes for herpes-simplex-virus-specific thymidine kinase, HSV-1-tk. The choice of the HSV-1-tk gene arose from the well-known application of acyclovir in the treatment of herpes simplex virus infection. Acyclovir is a purine analogue

(9-[(2-hydroxyethoxy)methyl]guanine) and a substrate for the HSV-1-tk but not for the more substrate-specific thymidine kinase in normal mammalian cells. After its phosphorylation acyclovir becomes substrate to host cell kinases to be phosphorylated to triphosphate. As such, it inhibits virus DNA polymerase and also may be incorporated into virus DNA with subsequent nucleotide chain termination. Nucleotides as phosphorylated substrates do not easily escape cells but remain trapped. An analogue of acyclovir, ganciclovir (9-[(1,3-dihydroxy-2-propoxy)methyl]guanine) is again a substrate of HSV-1-tk, yet also to a lesser extent for thymidine kinase in normal mammalian cells. The triphosphate of ganciclovir is toxic to any cell where it is formed and trapped. Thus, transfecting tumor cells first with the HSV-1 virus, and then exposing them to ganciclovir will overwhelmingly affect the tumor host cells. Since the HSV-1-tk is less substrate-specific than thymidine kinase in normal mammalian cells, it also accepts thymidine analogues as substrates for phosphorylation.

Therefore, two classes of substrates have been extensively studied to observe in vivo the expression of the HSV-1-tk gene by monitoring its transcription product: one is the group of guanine derivatives, and the other encompasses thymidine and its analogues, both labeled, for example, with ^3H for autoradiography, or with ^{123}I for single-photon-tomography, or ^{11}C or ^{18}F for PET. The advantage of ^{123}I , and ^{18}F is, of course, their relatively long physical half-life, which allows for delayed imaging. The guanine analogues used as indicator for HSV-1-tk activity include, for instance, labeled acyclovir (ACV), ganciclovir (GCV), fluoroganciclovir (FGCV), penciclovir (PCV), fluoropenciclovir (FPCV), 9-[3-fluoro-1-hydroxy-2-propoxymethyl] guanine (FHPG), and 9-[4-fluoro-3-(hydroxymethyl)butyl] guanine (FHBG). The thymidine analogue group includes ^{123}I - and ^{18}F -labeled indicators: 3'-deoxy-3'-fluorothymidine (FLT), 5 iodo-2'-deoxyuridine (5-IUdR), and the metabolically more stable 2'-fluoro-2'-deoxy-1-b-D-arabinofuranosyl-5-iodouracil (FIAU), 2'-fluoro-2'-deoxy-5-iodo-1-b-D-ribofuranosyl-uracil (FIRU), 2'-fluoro-2'-deoxy-5-methyl-1-b-D-arabino-furanosyl-uracil (FMAU), and 2'-fluoro-2'-deoxy-5-iodovinyl-1-b-D-ribofuranosyl-uracil (IVFRU). These and other similarly labeled substrate analogues are still under study and being compared in various cell systems in vitro and in vivo with different advantages and disadvantages regarding their metabolic fate in the whole organism, degree of

background signaling, and cell specificity in the context of using the HSV-1-tk as reporter gene in linkage with a primary gene and a common promoter for both in a DNA construct for transfer.

The choice of the DNA construct, or “cassette,” varies with the goal of the transfer. Besides the choice of primary gene for transfer, various reporter genes may offer advantages. In addition, the promoter may be chosen to be cell specific and exclude gene expression in certain cells. Or the promoter may function either continuously or upon a defined external signal such as a drug or low-dose irradiation.

Transfection of cells relies on various vectors well known in cell biology. They include a virus-type vector, liposomes, plasmids, or purposefully guiding peptides. Important is the proper binding of the vector to the cell membrane with subsequent internalization. These types of vector, as long as they operate, usually do not affect the functioning of the transferred gene complex.

Transfection may use cells in vitro to be transferred into body tissue, or may directly aim at cells in the body. For experimental and some therapeutic reasons, in vitro transfected cells are injected into the site of envisaged action. Reporter genes have thus been experimentally transferred to cells in different body sites and shown to function.

In summary, a gene or genetic construct with its reporter gene in a complying vector must enter the target cells to become integrated into the host genome or be metabolized rapidly. The transferred gene alone or its reporter gene should not already be present in the cells under observation and should be specific for the transfected cells. The expression of the primary gene or, if present, its reporter gene should transcribe a product that has a biological residence time long enough to be measured. In vivo imaging gains from an appropriately labeled indicator substrate to bind irreversibly to the gene product such as enzyme, receptor, or transporter, without disturbing cellular homeostasis. Of course, any of the above-mentioned methods for in vivo measuring a biochemical reaction may be useful.

No doubt the possibility of in vivo observing the expression of transferred genes, its duration, and location in cells and tissues will bring new technologies and insights and will immensely benefit clinical research and practice both in diagnosis and therapy. Molecular biology research and application in living tissues as complex adaptive systems is becoming a reality.

1.7

Conclusion

This condensed overview and review of the present state of art in measuring biochemical reactions as they occur in living systems presents the various approaches that allow the linking of gene expression to cell function at the molecular level. Justification, goals, complicating factors, and solutions are given in a synopsis. Some of these techniques are currently more in use than others, but the power of all is evident. The following chapters in this book give more details than the sections in this chapter, in the context of different research and clinical demands. Common to nearly all procedures is the multiple parameter analysis, whether it applies to dynamic imaging of whole body or its regions of interest, or to in vitro analyses of body fluids, excreta, and/or exhaled breath. The various parameters of measurement may combine tracers, substrates and tissue compartments. In whatever interrelationship, the simultaneous attention to different parameters appears to be nearly indispensable for in vivo quantification of biochemical reactions.

Increased investments in cost and labor into further developing these approaches appear to be worthwhile in that they promise to lead to the understanding of fundamental biological functions in living systems, in fact bring molecular biology and cell biology into intimate contact with clinical medicine. They allow the biomedical researcher and physician to respond to the challenge of in vivo functional genomics and proteomics and thus open new diagnostic and therapeutic dimensions.

1.8

References

- Antar MA, Spohr G, Herzog HH et al (1986) 15-(ortho-123-I-phenyl)-pentadecanoic acid, a new myocardial imaging agent for clinical use. *Nucl Med Commun* 7:683–696
- Armbrrecht JJ, Buxton DB, Schelbert HR (1990) Validation of [1-11-C]acetate as a tracer for noninvasive assessment of oxidative metabolism with positron emission tomography in normal, ischemic, postischemic, and hyperemic canine myocardium. *Circulation* 81:1594–1605
- Ballare E, Persana L, Lania AG et al (2001) Mutation of somatostatin receptor type 5 in an acromegalic patient resistant to somatostatin analog treatment. *J Clin Endocrinol Metab* 86:3809–3814
- Beckurts TE, Shreeve WW, Schieren R et al (1985) Kinetics of different ¹²³I- and ¹⁴C-labeled fatty acids in normal

- and diabetic rat myocardium in vivo. *Nuclear Med Commun* 6:415–424
- Bengel FM, Permanetter B, Ungerer M et al (2002) Alterations of the sympathetic nervous system and metabolic performance of the cardiomyopathic heart. *Eur J Nucl Med Mol Imaging* 29:198–202
- Blaufox MD (1996) Becquerel and the discovery of radioactivity: early concepts. *Semin Nucl Med* XXVI:145–154
- Boerman OC, Oyen WJG, Corstens FHM (2000) Radio-labeled receptor-binding peptides: a new class of radiopharmaceuticals. *Semin Nucl Med* 30:195–208
- Brown MA, Marshall DR, Sobel BE et al (1987) Delineation of myocardial oxygen utilization with carbon-11 labeled acetate. *Circulation* 76:687–696
- DeGrazia JA, Ivanovich P, Fellows H et al (1965) A double isotope method for measurement of intestinal absorption of calcium in man. *J Lab Clin Med* 66:822–829
- Delbeke D, Martin WH, Patton JA, Sandler M (eds) (2002) Practical FDG imaging, a teaching file. Springer, Berlin Heidelberg New York
- De Meutter RC, Shreeve WW (1963) Conversion of DL-lactate-2-C¹⁴ or 3-C¹⁴ or pyruvate-2-C¹⁴ to blood glucose in humans: effects of diabetes, insulin, tolbutamide and glucose load. *J Clin Invest* 42:525–533
- Ebert A, Feinendegen DL, Czech N et al (1993) Erfassung des Lipidstoffwechsels und der hepatozellulären Viabilität mittels 15-(para-123-J-Phenyl)-Pentadecansäure (pPPA) und 15-(ortho-131-J-Phenyl)-Pentadecansäure (oPPA) (abstract). *Nuklearmedizin* 32a:105
- Feinendegen LE (1993) Single photon metabolic imaging in cardiology. In: Zaret BL, Beller GA (eds) *Nuclear cardiology, state of the art and future directions*, chap 24. Mosby Year Book, St Louis, Mo
- Feinendegen LE (2000) Myocardial imaging of lipid metabolism with labeled fatty acids. In: Dilsizian V (ed) *Myocardial viability: a clinical and scientific treatise*, chap 16. Futura, Armonk NY
- Feinendegen LE, Ritzl F (1971) Insulin metabolism determination in vivo using iodine-125 and chromium-51 double labeling. *Nucl Med (Stuttg)* 9:748–751
- Feinendegen LE, Heiniger HJ, Friedrich G et al (1973) Differences in reutilization of thymidine in hemopoietic and lymphopoietic tissues of the normal mouse. *Cell Tissue Kinet* 6:573–585
- Feinendegen LE, Vyska K, Freundlieb C et al (1981) Non-invasive analysis of metabolic reactions in body tissues, the case of myocardial fatty acids. *Eur J Nucl Med* 6:191–200
- Feinendegen LE, Henrich MM, Kuikka JT et al (1995) Myocardial lipid turnover in dilated cardiomyopathy: a dual in vivo tracer approach. *J Nucl Cardiol* 2:42–52
- Feinendegen DL, Ohlenschlaeger U, Grossmann K et al (1996) Lipid metabolism in the liver studied in vivo with two isomers of labeled fatty acid analogs. *J Nucl Med* 37:1841–1845
- Feinendegen LE, Herzog H, Thompson KH (2001) Cerebral glucose transport implies individualized glial cell function. *J Cereb Blood Flow Metab* 21:1160–1170
- Firna G, Garnett ES, Chan PK et al (1976) Intracerebral dopamine metabolism studied by a novel radioisotope technique. *J Pharm Pharmacol* 28:584–585
- Fowler JS, Wolf AP, MacGregor RR et al (1988) Mechanistic positron emission tomography studies: demonstration of a deuterium isotope effect in the monoamine oxidase-catalyzed binding of {C-11}-deprenyl in living baboon brain. *J Neurochem* 51:1524–1534
- Freundlieb C, Hoeck A, Vyska K et al (1978) Use of ω -123-I-labeled heptadecanoic acids for non-invasively measuring myocardial metabolism. In: Woldring M, Schmidt HAE (eds) *Proceedings of the 15th international meeting of the Society of Nuclear Medicine*, Groningen, 1977. Schattauer, Stuttgart, pp 216–219
- Freundlieb C, Hoeck A, Vyska K et al (1980) Myocardial imaging and metabolic studies with (17-123-I) iodoheptadecanoic acid. *J Nucl Med* 21:1943–1950
- Friedrich G, Feinendegen LE, Heiniger HJ (1972) Studies on the incorporation of exogenous DNA in mammalian cells. *Hoppe Seylers Z Physiol Chem* 353:705–706
- Gallagher BM, Ansari A, Atkins H et al (1977) Radiopharmaceuticals XXVII. 18F-labeled 2-deoxy-2-fluoro-d-glucose as a radiopharmaceutical for measuring regional myocardial glucose metabolism in vivo: tissue distribution and imaging studies in animals. *J Nucl Med* 18:990–996
- Gjedde A, Diemer NH (1983) Kinetic analysis of the uptake of glucose and some of its analogs in the brain using the single capillary model: comments on some points of controversy. In: Lambrecht RM, Rescigno A (eds) *Lecture notes in biomathematics 48: Tracer kinetics and physiological modeling*. Springer, Berlin Heidelberg New York, pp 387–410
- Gruvberger S, Ringner M, Chen Y et al (2001) Estrogen receptor status in breast cancer is associated with remarkably distinct gene expression patterns. *J Cancer Res* 61:5979–5984
- Herzog H, Lele VR, Kuwert T et al (1990/1991) Changed pattern of regional glucose metabolism during yoga meditative relaxation. *Neuropsychobiology* 23:182–187
- Hevesy G (1913) Radioelements as indicators in chemistry and physics. *Chem News* 108:166–167
- Hevesy G (1948) *Radioactive indicators*. Interscience, New York
- Hoeck A, Spohr G, Schmitz M et al (1986) 17-iodine-123 iodoheptadecanoic acid for metabolic liver studies in humans. *J Nucl Med* 27:1533–1539
- Kaiser KP, Geuting B, Grossmann K et al (1990) Tracer kinetics of 15-(ortho-123-/131-I-phenyl)-pentadecanoic acid (oPPA) and 15-(para-123/131-I-phenyl)-pentadecanoic acid (pPPA) in animals and man. *J Nucl Med* 31:1608–1616
- Kallie RN, Shreeve WW, Joubert SM (1968) Studies in primary hyperuricaemia III. The conversion of ¹⁴C to ¹⁴CO₂ from glucose-1-¹⁴C and glucose-6-¹⁴C in hyperuricaemia and gout. *S African Med J* 42:473–476
- Kelly DP, Mendelsohn NJ, Sobel BE et al (1993) Detection and assessment by positron emission tomography of a genetically determined defect in myocardial fatty acid utilization (long-chain acyl-Co-A dehydrogenase deficiency). *Am J Cardiol* 71:738–744
- Knapp FF Jr, Kropp J (1995) Iodine-123-labeled fatty acids for myocardial single photon emission tomography: Current status and future perspectives. *Eur J Nucl Med* 22:361–381

- Kim EE, Yang DJ (eds) (2001) Targeted molecular imaging in oncology. Springer, Berlin Heidelberg New York
- Landau BR, Chandramouli V, Schumann WC et al (1995) Estimates of Krebs cycle activity and contributions of gluconeogenesis to hepatic glucose production in fasting health subjects and IDDM patients. *Diabetologia* 38:831–838
- Laruelle M (2000) Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. *J Cereb Blood Flow Metab* 20:423–451
- Logan J, Fowler JD, Volkow ND et al (1990) Graphical analysis of reversible radioligand binding from time-activity measurements applied to [*N*-11C-methyl]-(-)-cocaine PET studies in human subjects. *J Cereb Blood Flow Metab* 10:740–747
- Machulla H-J, Stoecklin G, Kupfernagel CH et al (1978) Comparative evaluation of fatty acids labeled with C-11, Cl-34m, Br-77, and I-123 for metabolic studies of the myocardium: concise communication. *J Nucl Med* 19:298–302
- Machulla H-J, Marsmann M, Dutschka K et al (1980) Biochemical concept and synthesis of a radioiodinated phenylfatty acid for in vivo metabolic studies of the myocardium. *Eur J Nucl Med* 5:171–173
- Maier BA (2001) Researchers focus on histone code. *Scientist* 15:15–16
- Meyers DK, Feinendegen LE (1975a) Incorporation of thymidine and iododeoxyuridine into the DNA of mouse tissues. *Can J Physiol Pharmacol* 53:1014–1022
- Meyers DK, Feinendegen LE (1975b) Double labeling with [3H]thymidine and [125I]iododeoxyuridine as a method for determining the fate of injected DNA and cells in vivo. *J Cell Biol* 67:484–488
- Meyers DK, Feinendegen LE (1976) DNA turnover and thymidine re-utilization in mouse tissues. *Cell Tissue Kinet* 9:215–221
- Nyhan WL (1984) Nonketotic hyperglycinemia. In: Nyhan WL (ed) Abnormalities in amino acid metabolism in clinical medicine, chap 34. Appleton-Century-Croft, New York, pp 333–351
- O'Brien KO, Zaveleta N, Caulfield LE et al (1999) Influence of prenatal iron and zinc supplements on supplemental iron absorption, red blood cell incorporation, and iron status in pregnant Peruvian women. *Am J Clin Nutr* 69:509–515
- Patlak CS, Blasberg RG, Fenstermacher JD (1983) Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. *J Cereb Blood Flow Metab* 3:1–7
- Phelps ME, Huang SC, Hoffman EJ et al (1979) Tomographic measurement of local cerebral glucose metabolic rate in humans with (F-18) 2-fluoro-2-deoxy-D-glucose: validation of method. *Ann Neurol* 6:371–388
- Rader DJ, Schaefer JR, Lohse P et al (1993) Increased production of apolipoprotein A-1 associated with elevated plasma levels of high-density lipoproteins, apolipoprotein A-1 and lipoprotein A1 in a patient with hyperalphalipoproteinemia. *Metab Clin Exp* 42:1429–1434
- Reivich M, Kuhl D, Wolf A et al (1979) The (¹⁸F)fluorodeoxyglucose method for the measurement of local cerebral glucose utilization in man. *Circ Res* 44:117–127
- Rensberger B (1996) Life itself: exploring the realm of the living cell. Oxford University Press, Oxford
- Ritzl F, Feinendegen LE (1971) In vivo determination of site and rate of insulin catabolism using the double tracer technique with 51-Cr and 131-I. In: Dynamic studies with radioisotopes in medicine. Proceedings of the Symposium on Dynamic Studies with Radioisotopes in Clinical Medicine and Research. International Atomic Energy Agency, Vienna, Austria, pp 57–68
- Ritzl F, Feinendegen LE, Schnipper HG (1974) A double isotope technique for estimating insulin degradation in vivo. *Nucl Med (Stuttg)* 13:85–97
- Ritz P, Coward WA (1995) Doubly labeled water measurement of total energy expenditure. *Diabetes Metab* 21:241–251
- Rodwell VW (1996) Enzymes: kinetics. In: Murray RK, Granner DK, Mayes PA et al (eds) Harper's biochemistry, 24th edn, chap 9. Appleton and Lange, Stamford, Conn, pp 75–90
- Sasaki Y (1995) Carbon-14 and Carbon-13 breath tests. In: Wagner HN Jr, Szabo Z, Buchanan JW (eds) Principles of nuclear medicine, 2nd edn, chap 40. Saunders, Philadelphia, pp 958–965
- Schelbert HR, Henze E, Sochor H et al (1986) Effects of substrate availability on myocardial C-11 palmitate kinetics by positron emission tomography in normal subjects and patients with ventricular dysfunction. *Am Heart J* 111:1055–1064
- Schoenheimer R (1946) The dynamic state of body constituents. Harvard University Press, Cambridge, Mass, pp 1–78
- Schroeder H, Schelbert HR (2000) Positron emission tomography for the assessment of myocardial viability: noninvasive approach to cardiac pathophysiology. In: Dilsizian V (ed) Myocardial viability: a clinical and scientific treatise, chap 17. Futura, Armonk, NY
- Schultze B, Gregoire F, Hughes WL (1964) Renal uptake of pancreatic ribonuclease after intravenous injection in mice and rats. Technical Report, Brookhaven National Laboratory, Upton, NY, BNL-8683
- Shapiro B, Gross MD, Sisson JS (1995) Neural crest tumors. In: Wagner HN Jr, Szabo Z, Buchanan JW (eds) Principles of nuclear medicine, 2nd edn, chap 33. Saunders, Philadelphia, pp 665–680
- Shreeve WW, Cerasi E, Luft R (1970) Metabolism of (2-¹⁴C) pyruvate in normal, acromegalic and growth hormone-treated human subjects. *Acta Endocrinol* 65:155–169
- Shreeve WW, Tashjian AJ, Oji N et al (1971) Formation of ¹⁴CO₂ and ³HOH from glucose-1-¹⁴C-1-³H during oral cortisone glucose tolerance tests in obese patients. *Metab Clin Exp* 20:280–292
- Shreeve WW, Schieren R, Machulla HJ et al (1984) Hepatic uptake and fate of ¹²³I- and ¹⁴C-fatty acids in normal and ethanolic mice. *Nucl Med Commun* 5:519–524
- Silverman DHS, Hoe CK, Seltzer MA et al (1998) Evaluating tumor biology and oncological disease with positron-emission tomography. *Semin Rad Oncol* 8:183–196
- Smith SM, Wastney ME, Nyquist LE et al (1996) Calcium kinetics with microgram stable isotope doses and saliva sampling. *J Mass Spectrom (CMB)* 31:1265–1270

- Sokoloff L, Reivich M, Kenney C et al (1977) The [14C]deoxyglucose method for the measurement of focal cerebral glucose utilization: theory, procedure and normal values in the conscious and anesthetized albino rat. *J Neurochem* 28:897–916
- Stern MP (2000) Strategies and prospects for finding insulin resistance genes. *J Clin Invest* 106:323–327
- Stryer L (1995) Enzymes: basic concepts and kinetics. In: Stryer L, *Biochemistry*, 4th edn, chap 8. Freeman, New York, pp 181–206
- Syrota A, Merlet P, Delforge J (1995) The heart: clinical neurotransmission. In: Wagner HN Jr, Szabo Z, Buchanan JW (eds) *Principles of nuclear medicine*, 2nd edn, chap 37, sect 2. Saunders, Philadelphia, pp 759–773
- Taylor SI (1995) Diabetes mellitus. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) *The metabolic and molecular bases of inherited disease I*, 7th edn, chap 21. McGraw-Hill, NY, pp 843–896
- Van Eenige MJ, Visser FC, Duwel CMB et al (1987) Analysis of myocardial time activity curves of I-123-heptadecanoic acid I. Curve fitting. *Nucl Med* 26:241–247
- Wagner HN Jr (1995) Nuclear medicine: what it is, what it does. In: Wagner HN Jr, Szabo Z, Buchanan JW (eds) *Principles of nuclear medicine*, 2nd edn, chap 1. Saunders, Philadelphia, pp 1–8
- Wagner HN, Burns HD, Dannals RF et al (1983) Imaging dopamine receptors in the human brain by positron tomography. *Science* 221:1264–1266
- Wagner HN Jr, Szabo Z, Buchanan JW (eds) (1995) *Principles of nuclear medicine*, 2nd edn. Saunders, Philadelphia

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