THE UNIVERSITY OF NEW MEXICO
HEALTH SCIENCES CENTER
COLLEGE OF PHARMACY
ALBUQUERQUE, NEW MEXICO

Correspondence Continuing Education Courses For Nuclear Pharmacists and Nuclear Medicine Professionals

VOLUME X, NUMBERS 3 AND 4

The Role of Gamma Scintigraphy in the Study of Drug Delivery

By:
Malcolm Frier, BPharm, FRPharmS, PhD and
Alan C. Perkins, BSc, MSc, PhD, SRCS, FIPEM, ARCP
Queens Medical Centre, Nottingham
University Hospital NHS Trust
Nottingham, UK

The University of New Mexico Health Sciences Center College of Pharmacy is approved by the American Council on Pharmaceutical Education as a provider of continuing pharmaceutical education. Program No. 039-000-02-005-H04. 6.0 Contact Hours or .60 CEUs.

PLEASE NOTE:

This is a double lesson: Volume 10, Lessons 3 and 4. Together these lessons are worth a total of 0.6 CEUs (6.0 credit hours).

- You must complete and return two answer sheets in order to obtain full credit.
- Submit one answer sheet per lesson.
- There are 30 questions per lesson.
- Complete one answer sheet for Lesson 3. Questions 1-30.
- Complete one answer sheet for Lesson 4. Questions 31-60.

The Role of Gamma Scintigraphy in the Study of Drug Delivery

By:

Malcolm Frier, BPharm, FRPharmS, PhD and
Alan C. Perkins, BSc, MSc, PhD, SRCS, FIPEM, ARCP

THE ROLE OF GAMMA SCINTIGRAPHY IN THE STUDY OF DRUG DELIVERY
STATEMENT OF OBJECTIVES

The primary purpose of this lesson is to increase the reader's knowledge base of the potential offered by gamma scintigraphy in the evaluation of drug delivery systems. The lesson describes the ethical and technical background relating to the performance of such studies in human subjects. Specific examples of drug delivery are presented, which demonstrate the ability of the technique to reveal information about the roles of formulation and physiology in the behavior of drug delivery systems.

Upon completion of this material, the reader should be able to:

1. Discuss of the relevant ethical and regulatory controls relating to the conduct of scintigraphic drug delivery studies on human subjects;
2. Explain the use of instrumentation required for the performance of scintigraphic drug delivery studies;
3. Describe the ways in which the principles of current Good Manufacturing Practices (cGMP apply to the manufacture of dosage forms containing gamma-emitting radionuclides);
4. Discuss the nature and extent of validation required to allow subsequent interpretation of scintigraphic data in terms of formulation behavior;
5. Explain how the technique can be applied to the study of the gastrointestinal tract
6. Describe how the technique can be applied to the study of the eye and the lung;
7. Clarify the differences between scintigraphic drug delivery studies and studies of drug biodistribution and metabolism using radiolabeled drug molecules; and
8. Describe the capabilities and limitations of planar imaging and single photon emission computed tomography (SPECT) in comparison with positron emission tomography (PET).

COURSE OUTLINE

I. INTRODUCTION
II. ETHICAL AND TECHNICAL CONSIDERATIONS
  A. Ethical and Regulatory Controls
    1. Radioactive Drug Research Committee
    2. Good Clinical Practices
    3. The Trial Protocol
    4. Institutional Review Board/Institutional Ethics Committee
    5. Investigators
    6. Data Handling and Analysis
    7. Quality Management
    8. Radiation Dosimetry
    9. Current Good Manufacturing Practices
  B. Instrumentation, Imaging, and Data Analysis
    1. Basic Technology
    2. Data Acquisition Format
    3. Data Analysis
    4. PET Imaging
    5. Quality Assurance
III. SCINTIGRAPHIC DRUG DELIVERY STUDIES
  A. The Gastrointestinal Tract
    1. Radiopharmaceuticals
    2. Esophageal Transit and Retention of Dosage Forms
    3. Radiolabeled Controlled-Release Formulations and Targeted Drug Delivery
    4. Imaging and Presentation of the Data
    5. Value of Studies in Formulation Design
    6. Case Study of Radiolabeled Solid Oral Dosage Formulations
  B. The Eye
    1. In Vivo Methods for Assessment of Ocular Retention
    2. Scintigraphic Studies
    3. Radiolabeling of Ocular Formulations
    4. Particulate Suspensions
    5. Non-Aqueous Vehicle Systems
    6. Imaging Procedure
    7. Data Analysis
    8. Results
    9. Summary
  C. The Lung

Unit Conversion Table

RADIATION

Absorbed Dose:	100 rad	=	1 Gy
Dose Equivalent:	100 rem	=	1 Sv
Activity:	1 Ci	=	3.7×10^{10} dps = 37 GBq
	1 Bq	=	1 dps = 27 pCi

$$1\frac{Sv}{Bq} = 3.7\times10^{12}\ \frac{rem}{Ci}$$

$$1\frac{(mrem/yr)}{(\mu Ci/m^2)} = 0.114\ \frac{(rem/hr)}{(Ci/m^2)}$$

$$1\frac{\mu Ci}{kg} = 1000\frac{pCi}{g}$$

$$1\frac{Ci}{m^3} = 1\frac{\mu Ci}{cm^3} = 1\frac{mCi}{\ell}$$

$$1\frac{\mu Ci}{m^2} = 1\frac{Ci}{km^2} = 100\ \frac{pCi}{cm^2}$$

Dose Equivalent

rem	Sievert
0.1 mrem	1 µSv
1 mrem	10 µSv
10 mrem	100 µSv (0.1 mSv)
100 mrem	1 mSv
500 mrem	5 mSv
1 rem	10 mSv
5 rem	50 mSv
10 rem	100 mSv
25 rem	250 mSv
50 rem	500 mSv
100 rem	1 Sv

Absorbed Dose

100 rad = 1 Gy (gray)

Activity

Curie	Becquerel
1 pCi	37 mBq
27 pCi	1 Bq
1 nCi	37 Bq
27 nCi	1 kBq
1 µCi	37 kBq
27 µCi	1 MBq
1 mCi	37 MBq
27 mCi	1 GBq
1 Ci	37 GBq
27 Ci	1 TBq
1 kCi	37 TBq
27 kCi	1 PBq
1Mci	37 PBq

THE ROLE OF GAMMA SCINTIGRAPHY IN THE STUDY OF DRUG DELIVERY

By: Malcolm Frier, BPharm, FRPharmS, PhD and Alan C. Perkins, BSc, MSc, PhD, SRCS, FIPEM, ARCP
Radiopharmacy Unit, Department of Medical Physics and Therapeutics, University Hospital, Queen's Medical Centre, Nottingham, United Kingdom.

INTRODUCTION

Nuclear medicine images are primarily functional in nature, providing substantial information on physiology and more limited information on anatomy. The technique, commonly termed scintigraphy, involves imaging the biodistribution of a radiopharmaceutical. Gamma camera images of the in vivo distribution of conventional pharmaceutical formulations, radiolabeled with a suitable gamma emitting radionuclide, may be used to quantify the biodistribution of formulations and the release and kinetics of drug delivery from novel carrier systems and devices. The strength of the technique lies in the quantitative nature of scintigraphic images. No other technique can locate so precisely the site of disintegration of a tablet in the GI tract, the depth of penetration of a nebulized solution into the lung, or the residence time of a drug on the cornea. Scintigraphic techniques also allow correlation between observed pharmacological effects and the precise site of delivery, thus facilitating drug-targeting studies.

Scintigraphic studies provide data on the nature and characteristics of products, such as reliability and reproducibility. They also offer the advantage of being performed in the very groups of patients intended to receive the dosage forms therapeutically since the presence of a disease condition can have a marked effect on physiology. Scintigraphic studies on drug delivery have been accepted by regulatory authorities as supporting evidence in product registration dossiers such as Investigational New Drug applications (INDs) or New Drug Applications (NDAs).

ETHICAL AND TECHNICAL CONSIDERATIONS

Ethical and Regulatory Controls

Ethical and regulatory controls in research involving human subjects are intended to encourage research without compromising high standards for health and safety. In recent years, regulatory agencies have begun to apply the practice of risk assessment[1] and cost-benefit analysis[2] for control purposes. Some countries are more highly advanced in this process regarding the extent to which these principles are used for the design of regulations involving health, safety, and the environment. Over the last decade, the development of innovations in risk assessment has promised not only improvement in the scientific treatment of risk but more analytical approaches to risk management, resulting in more consistent human protection policies.

The approval of human studies in research and clinical trials encompasses both ethical and regulatory factors. Control of risk may be achieved in a variety of ways. Regulation had been the most widely used risk management tool, but more recently, non-regulatory options have received considerable attention, including economic, advisory, or technical solutions. Of the different options available, regulation remains the most widely used for clinical trials. The morals and principles applied to the conduct of medical or pharmaceutical research will only be as ethical as the current moral and ethical limitations placed on us by

society and by the extent of education and knowledge.

Research using radiopharmaceuticals is subject to the same ethical scrutiny as other drugs; however, the highly sensitive issue of radiation exposure to the patient adds additional criteria to the research parameters that must be evaluated *a priori*. The use of well-characterized radiopharmaceuticals in human studies is subject to less critical examination than novel, new chemical or biological entities labeled with novel radionuclides. Basic radiopharmaceutical research is used to obtain information regarding the metabolism (including kinetics, distribution and localization) of the radioactive drug or to investigate human pathology, pathophysiology, or biochemistry. The essence of a clinical trial is that there is some immediate clinical application in either diagnosis or therapy.

Drugs used in clinical trials are subject to the requirements of the regulatory agency in the country in which the trial will be performed. In the United States, the pertinent agency is the Food and Drug Administration (FDA). In addition, the requirements for drug research in humans may also vary from one regulatory agency to another and from study to study. These requirements have been reviewed.[3] Clinical trials designed to determine the safety and efficacy of a new drug have special requirements and, in the U.S., are located in the FDA regulations of 21 CFR Part 312. Studies in humans from which the resulting data will not be used in support of the drug approval process are also regulated by the FDA, but are subject to different requirements. Research involving drug delivery may fall under either category, depending on the intended use of the study results.

Radioactive Drug Research Committee

In the United States, drug delivery studies that utilize radioactive drugs may be subject to approval by a Radioactive Drug Research Committee (RDRC), at least at those institutions that have such a committee. An RDRC operates under the authority of the FDA and the regulations governing the RDRC are found in 21 CFR Part 361. The function of an RDRC is to facilitate, on a local level, approval of a research project involving the administration of a radioactive drug to human research subjects, specifically when the aim of the research project is "to obtain basic information regarding the metabolism (including kinetics, distribution, and localization) of a radioactively labeled drug or regarding human physiology, pathophysiology, or biochemistry." However, the RDRC will not consider such research projects if they are intended for "immediate therapeutic, diagnostic, or similar purposes, or to determine the safety and effectiveness of the drug in humans for such purposes (i.e., to carry out a clinical trial)." In other words, investigators (who might be seeking to avoid direct contact with the FDA) may not seek approval from an RDRC for the purpose of collecting safety and efficacy data that may eventually be used to support a New Drug Application (NDA).

In its consideration for approval of a specific research project, the RDRC must, among other things, assure that (1) both the pharmacological dose and the radiation dose of the radioactively labeled drug are within the limits set forth in the regulations, (2) the radiation exposure is justified by the quality of the study being undertaken and the importance of the information it seeks to obtain, (3) the study meets certain other

requirements set forth in the regulations regarding: (a) qualifications of the investigator, (b) proper licensure for handling radioactive materials, (c) selection and consent of research subjects, (d) the quality of radioactive drugs used, (e) research protocol design, (f) reporting of adverse reactions, and (g) approval by an appropriate Institutional Review Board.

Good Clinical Practice

Efforts to establish consistent approaches by regulatory bodies from different countries have been pursued through the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Within this organization, an Expert Working Group has developed Guidelines for Good Clinical Practice (GCP). The ICH GCP Guidelines,[4] that are based on thirteen principles, have been recommended for adoption by the regulatory bodies of the United States, the European Union, Japan, and Canada. These principles are as follows:

Clinical trials should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki,[5] and that are consistent with GCP and the applicable regulatory requirements.

Before a trial is initiated, foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial subject and society. A trial should be initiated and continued only if the anticipated benefits justify the risks.

The rights, safety, and well being of the trial's subjects are the most important considerations and should prevail over interests of science and society.

The available non-clinical and clinical information on an investigational product should be adequate to support the proposed clinical trial.

Clinical trials should be scientifically sound, and described in a clear, detailed protocol.

A trial should be conducted in compliance with the protocol that has received prior institutional review board (IRB) or independent ethics committee (IEC) approval.

The medical care given to, and medical decisions made on behalf of, subjects should always be the responsibility of a qualified physician or, when appropriate, of a qualified dentist.

Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).

Freely given informed consent should be obtained from every subject prior to clinical trial participation.

All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation, and verification.

The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

Investigational products should be manufactured, handled, and stored in accordance with applicable current good manufacturing practices (cGMPs). They should be used in accordance with the approved protocol.

Systems with procedures that assure the quality of every aspect of the trial should be implemented.

These principles refer to certain key elements, which will be described in the following sections.

Note: The website found at http://www.fda.gov/oc/gcp/ offers a wide range of information on Good Clinical Practice in FDA-regulated clinical trials, and this source is highly recommended for further details on GCP. Standards for clinical research within the NIH (National Institutes of Health) intramural research program are located on the Internet at http://www.cc.nih.gov/ccc/clinicalresearch/standards1.html. In addition, the website at the National Institute of Arthritis and Musculoskeletal and Skin Diseases provides useful links to various NIH policies for monitoring clinical research. http://www.niams.nih.gov/rtac/clinical/links.htm

The Trial Protocol

The trial protocol should be clear and sufficiently detailed to allow for review by the IRB/IEC and allow for organized progression of the study. Some site-specific information may be included in other protocol-referenced documents such as the investigator's brochure. The following elements should be included:

General Information

The protocol should have a title, an identification number, and a date. Amendments likewise should have amendment numbers and dates. The protocol should also identify the trial sponsor, monitor, authorized signatories, medical experts, the qualified study physician, and investigators responsible for the conduct of the study. Clinical and technical laboratories or other involved institutions should be identified.

Background Information

This should include the name and description of any investigational products, together with a summary of relevant findings from any relevant clinical or non-clinical studies. Relevant literature and background data should be cited. There should be a summary of known and potential risks and benefits, and descriptions of and justifications for the route of administration, dosage and dosage regimen, and treatment period. It is important to include a statement to the effect that the trial will be conducted in accordance with the protocol, Good Clinical Practice, and in compliance with all relevant regulatory requirements.

Objectives, Purpose and Design

Trial design is important in retaining the scientific integrity of the trial, and the credibility of the data. Good design requires a clear definition of objectives and purpose, and specific statements of the endpoints, both primary and secondary, to be measured. Good trial design, whether double blind, parallel or placebo controlled, requires careful consideration of measures to be adopted to avoid bias, including randomization, blinding, as well as the protection of the randomization codes, and the code-breaking procedures.

Subject Selection, Withdrawal and Treatment

Definitions are required for subject inclusion and exclusion, and withdrawal criteria. The latter should define when and how to withdraw subjects, data to be collected from withdrawn subjects, and follow-up for withdrawn subjects. Information on treatment should include administered medication, permitted rescue medication and procedures for monitoring subject compliance.

Efficacy, Safety, Outcomes and Data Analysis

Efficacy parameters and methods for their measurement, recording, and analysis must be carefully defined or specified. In like manner, protocol-referenced documents should be specified, including the recording and reporting of adverse events and follow-up procedures. A description of statistical methods should be included, bearing in mind the numbers of subjects, choice of sample size, clinical justification and power of the study. Statistical methods should be described in sufficient detail to allow reviewers to determine the appropriateness of these methods and the trial itself.

Monitoring and Audit

It is important that the protocol includes specifications to the effect that audit, IRB/IEC review, or regulatory inspection of source data or documents be permitted.

Institutional Review Board/Independent Ethics Committee

In the United States, regulations that detail information on protection of human subjects and Institutional Review Boards are located in 21 CFR Parts 50 and 56, respectively.

Composition, Function and Operation

The composition of the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) should be such that the collective experience is adequate to review and evaluate the science, medical aspects, and ethics of the proposed trial. A membership of five is suggested, at least one representative should be a lay member, and another should be independent of the investigation/trial site. Membership of the group should be recorded, together with minutes of all its actions, and all its functions performed in accordance with written operating procedures. Non-members with specific expertise can be invited to provide assistance; this can be particularly relevant to studies involving the use of ionizing radiations.

Responsibilities and Procedures

The responsibilities of the IRB/IEC can be summarized in the single statement that it exists to safeguard the rights, safety, and well being of all trial subjects. In order to exercise this responsibility, access is required to the following documents:

- Trial protocols and amendments,
- Written informed consent forms,
- Recruitment procedures including advertisements,
- Written information to be provided to the subjects,
- Investigators brochure,
- Available safety information,
- Information about payments and compensations available to trial subjects, and
- Curriculum vitae of the investigators.

Examination of this information will allow the IRB/IEC to review the proposed conduct of a trial with the assurance that it will be properly performed by appropriately qualified investigators, and that the rights and safety of trial subjects will be properly respected.

Investigators

In order to assume responsibility for the proper conduct of any study, it is necessary for the investigators to be properly qualified by education, training, and experience, including a thorough knowledge

of Good Clinical Practices, and other applicable regulatory requirements. The duties of each investigator within the trial should be defined and documented. All trial-related medical decisions should be the responsibility of a qualified physician. It is the responsibility of the Principal Investigator to ensure that the trial is properly conducted in accordance with the approved written protocol.

Data Handling and Analysis

Proper management of a trial depends on the utilization of appropriately qualified individuals. This includes those involved in the handling and verification of data, and in the application of appropriate methods for data analysis. It may be appropriate to appoint an independent group for data monitoring. Systems for electronic acquisition, storage, and analysis of data should be properly validated and maintained, and operated under standard operating procedures. Statistical methods to be employed should be described, including information on the levels of significance to be used, the power of the study, and procedures to account for missing, unused, or equivocal data. All source data and documents must be retained.

Quality Management

Sponsor's Duties

Trial sponsors have responsibility for implementing and maintaining systems of quality assurance and quality control, with written standard operating procedures to ensure the proper conduct of trials. They also have responsibility for ensuring agreement for all involved parties at all involved sites to allow access for the purposes of monitoring, auditing, and inspection.

Monitoring

It is essential that an appropriately trained monitor with the necessary scientific and clinical knowledge scrutinize the conduct of all trials. A monitor is required to be familiar with the protocol, the principles of Good Clinical Practice, and with appropriate regulatory requirements. The prime responsibilities of a monitor are to ensure that the trial is conducted in accordance with the approved written protocol, and that it is properly documented.

Auditors

Implementation of a quality assurance program may require the performance of an audit. If, and when, this is done, it should be performed by appropriately trained, qualified auditors who are independent of the clinical trial program.

Radiation Dosimetry

Exposure of participants in research studies to ionizing radiations raises a number of procedural and ethical questions. In submitting the research protocol to the IRB/IEC, a maximum effective dose to the individual completing the study must be stated. Good estimates of the absorbed radiation dose can be made when information on the radionuclide properties and decay schemes, the amount of radioactivity administered, and the biological fate of the radiopharmaceutical are known. Current knowledge of radionuclide properties is adequate. Biological data may be limited when radiopharmaceuticals are administered via novel routes, or in association with investigational products.

The amount of radioactive material to be administered should be such that the subject receives the smallest particle radiation dose. This target dose, generally

quoted as an effective dose equivalent, places the proposal in one of four categories identified by the International Commission for Radiological Protection (ICRP) in terms of cancer risk (see Table 1).[6]

Table 1: **The Relation of Effective Dose and Cancer Risk**

Dose Category	Range of Effective Dose (mSv)	Approximate Cancer Risk (ICRP60, general population)
A	Up to 0.2	Up to 1 in 100,000
B	More than 0.2 up to 2	Up to 1 in 10,000
C	More than 2 up to 20	Up to 1 in 1000
D	More than 20	Higher than 1 in 1,000

The limits for radiation dose set by the FDA in the RDRC regulations (21 CFR Part 361) are as follows:

- For the whole body, active blood-forming organs, lens of the eye, and gonads
  - Single dose 3 rem (30 mSv)
  - Annual and total dose commitment 5 rem (50 mSv)
- For other organs
  - Single dose 5 rem (50 mSv)
  - Annual and total dose commitment 15 rem (150 mSv)
- For research subjects under the age of 18
  - The radiation dose shall not exceed 10% of the above-stated limits.

In many cases, the volunteer participating in a trial involving a drug-delivery system will not gain direct benefit from the study. Therefore, any risks involved are those assumed by the individual, and it is important to explain the magnitude of the risk in a meaningful way such that proper informed consent may be obtained. It is equally undesirable to present too high or too low a perception of risk.

Risk can be presented in a similar way to the IRB/IEC. The principle currently being adopted by many regulatory authorities is that once the proposal, including information on radiation dosimetry, has been approved, then the target dose becomes the dose constraint required by law.

Good Manufacturing Practices

Adherence to the principles of current Good Manufacturing Practices (cGMPs) is required for the manufacture of investigational medicinal products. This will ensure their proper composition, characterization, coding and labeling, and the maintenance of appropriate blinding requirements. Drug delivery studies may involve the manufacture of delivery devices or dosage forms from basic raw materials, or the modification of previously manufactured devices or dosage forms. Strict adherence to standard operating procedures and careful characterization is essential during the manufacture of investigational products, especially if small batch preparation is performed using non-routine production methods.

Presently, medicinal products intended for investigational purposes are not the focus of marketing legislation within the European Community, but implementation

of Directive 2001/20/EC of the European Parliament will require prior authorization for their manufacture assembly or importation. In the United States, investigational drugs may not be distributed or imported for trial on humans unless the sponsor has filed an acceptable investigational new drug application (IND) as required by the FDA (Refer to 21 CFR Part 312). It is generally agreed that compliance is required with the principles of current Good Manufacturing Practices (cGMPs) during the manufacture of products intended for use in clinical trials. This is based on the suggestion that it is illogical for experimental products to be exempt from the controls that would apply to the formulations of which they are the prototypes.

Current Good Manufacturing Practices (cGMP) regulations for the United States are located in 21 CFR Parts 210 and 211. Certain aspects of cGMPs will be highlighted in this section as they apply to clinical investigation of drug delivery using scintigraphic methods. It is recognized that manufacture of investigational products may not take place under a set routine, and that there may be incomplete characterization of the product.

Application of cGMP principles may raise certain logistic problems because of the requirement to introduce radioactive, often short-lived, tracers into pharmaceutical dosage forms. Commercial pharmaceutical manufacturers are generally able to produce small batches of non-radioactive investigational products in a dedicated or pilot-scale plant, under conditions very similar to, if not identical to, those used in full-scale manufacture. However, it is rare to find commercial manufacturers with on-site production facilities for the manipulation of radioactive materials. In these circumstances, dosage forms must be modified after manufacture in premises not under the direct control of the manufacturer.

There may be circumstances in which the whole of the manufacturing process must be outsourced to sites appropriately authorized to handle radioactive substances. Techniques for the incorporation of radionuclides vary in complexity. Techniques range from simple physical manipulation of pre-formed tablets, to complete fabrication of complex dosage forms including metered-dose inhalers[9] and controlled-release capsules.[10] Post-production radiolabeling using neutron activation offers distinct advantages in the achievement of cGMP standards by permitting manipulation of non-active components and products in dedicated or pilot scale facilities, with subsequent irradiation of the complete product.

Elements of Good Manufacturing Practices

Good Manufacturing Practices are that part of quality assurance that ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use. They are concerned with both production and quality control, and require the implementation of a highly effective quality management system, which assumes greater significance in the manufacture of investigational medicinal products, because of the non-routine nature or increased complexity of the operations involved. The quality management system should extend to cover the following main areas.

Manufacturing Site

Any location in which investigational product manufacture takes place should be secure and provide a

constant, controlled environment. It is very important to have appropriate procedures in place to avoid product mix-up, and cross-contamination. The site should be cleaned using appropriate, written and recorded procedures, and an operational log of all procedures performed within the premises should be maintained.

Personnel

Any staff involved in preparation procedures should be appropriately qualified and trained, and the training should include formal and documented instruction in cGMPs. The nature of investigational manufacture is such that the numbers of staff involved will be small, although ideally, there should be separate people involved in production and quality control. This may not always be possible, but at the very least, release procedures should be established prior to the start of the study, and at every step in the manufacturing process, two members of staff should be involved to provide independent verification.

Protocols

It is essential that starting materials and finished products be clearly specified in protocols, which also include criteria for release of the finished product. Processing instructions need to be in writing, and provision made for recording of batch manufacturing details. However, it is recognized that the product specifications and processing instruction may vary during the developmental stages, and this is quite acceptable provided the rationale is explained, and any that changes are authorized and recorded.

Processing

All processing should be performed in accordance with written standard operating procedures (SOPs). Variations in procedure are permissible, but prior authorization should be sought, and the nature of the variation, together with the reasons, documented in a SOP deviation report. Validation of procedures is crucial, since, by their nature, they are investigational and non-routine. Validation can be direct, or in some cases may have to be performed parametrically as, for example, when assessing the effect of a radiolabeling procedure on a pharmaceutical product. It is often convenient to test the process by performing the required manipulations in the absence of the radioactive component.

Manufacturing Plant and Equipment

As for processing, the keyword here is validation. Any equipment or plant used must perform reliably within known parameters, and it is usual to carry out validations at three stages, namely on installation, during routine operation, and during performance of the actual process in question. Validation at installation is simply to confirm that the equipment conforms to its specifications. Validation during operation establishes the working parameters, and it is expected that measurements taken during performance of the actual procedure will fall within those parameters. Appropriate cleaning procedures are essential not only to avoid cross contamination, but also to ensure that materials used in cleaning do not have a detrimental effect on the products themselves. It is therefore essential that cleaning procedures be validated, documented, and recorded. Equipment including balances and ionization chambers must be calibrated using test weights or radioactive sources with certification traceable to national standards. Usage of

equipment is continually recorded in the form of an operational log.

Products

It is usual to estimate the yield of any production process. In the context of preparing a radioactively labeled product, this will usually take the form of an estimate of radioactivity, and will need to take into account time differences between preparation and dosing, to compensate for radioactive decay (i.e., decay corrected). Actual and theoretical yields should be reconciled, and any discrepancies investigated, described, and documented. As previously described, product release is only permitted in accordance with a standard operating procedure which specifies the release criteria. If storage is required between preparation and use, the storage area should be secured, dedicated or segregated, and monitored for appropriate conditions such as temperature and humidity. These storage areas should also be inspected or audited periodically for compliance with study requirements. Issuance of study or test material must be upon written direction and appropriate records maintained. Surplus or unused materials may only be disposed of or destroyed in accordance with written standard operating procedures.

Neutron Activation

An elegant approach to radio-labeling that also addresses many issues related to cGMPs, is to incorporate the non-radioactive oxide of samarium-152 (^{152}Sm), erbium-170 (^{170}Er), or ytterbium-174 (^{174}Yb) into the formulation during manufacture. This will permit subsequent neutron activation in a nuclear reactor to produce the radioactive products ^{153}Sm, ^{171}Er, or ^{175}Yb, respectively, which may be used for imaging.[11-13] Both samarium and erbium oxide are non-absorbable within the gastrointestinal tract and are suitable for studying slow-release oral drug formulations. These techniques allow manufacture of products in facilities that comply with cGMPs since no handling of radioactive material is involved until the final irradiation. They also address the situation where production processes are lengthy or consist of multiple stages; for example, the production of multi-layered compressed tablets, or sugarcoated formulations, since they avoid the problem of lengthy handling of radioactive products, or of excessive radioactive decay occurring during the production process. It is still necessary to consider the quality of source materials, particularly of the erbium and samarium oxides themselves, and validation is crucial since significant changes can be induced by the irradiation procedure itself, which can bring about substantial heating of the target material.

Compliance with cGMPs during Radiolabeling Procedures

There are several areas where compliance with cGMPs introduces difficulties in radiolabeling procedure. One simple example is when tablets or capsules must be sent off site for neutron activation. During this time, conditions of storage and handling are outside the control of the manufacturer. Procedures need to be audited prior to commencement of any batch manufacture to assess factors such as temperature during irradiation, and mechanical damage due to loading and transport. Security and integrity of the product should be assured by enclosing the products in tamper-proof or tamper-evident closures during the whole procedure.

Conformity with cGMPs can often dictate that products are radiolabeled some time in advance of the required time for dosing. If working with short-lived tracers, this can mean handling significant levels of radioactivity during the labeling procedure, and radiation protection becomes a major concern. For example, it is practical to introduce 5 mg of a powder containing 5 MBq of ^{99m}Tc activity into a drilled tablet. If this operation is performed 24 hours in advance, each 5 mg of powder must contain 80 MBq of ^{99m}Tc radioactivity. For practical considerations, it is feasible to handle about 200 mg of the powder at the bulk labeling stage, which translates into a total requirement of 3.2 GBq.

There is no simple description of "Good Manufacturing Practices." The most important concept is that they are not seen as something "extra" added to a process, rather that they are the process, implicit at every stage involved in the preparation of pharmaceutical products for use in clinical trials. Seen from the regulatory affairs viewpoint, adoption of the principles of Good Manufacturing Practices, Good Clinical Practices, and Good Laboratory Practices is now essential to the acceptability of any supporting data included in product dossiers (information submitted for product approval). Adoption of the principles from the very outset helps prevent later compromise in the study design, and gives a high level of confidence in the quality of any results obtained.

Regulatory and ethical controls exist to protect the welfare and interests of the study subject while at the same time ensuring that the study will be viable. There is no intent to create impediments to research and at all costs, unjustified impediments should be avoided. The results of human research can often advance knowledge, relieve suffering and promote welfare. Impediments to research may be as unethical such as those who violate the dignity and safety of human subjects. Radiopharmaceuticals have a very special role to play in research. In a non-invasive manner, radiopharmaceuticals can be used to produce kinetic information on drugs and drug delivery systems and devices or can enhance our knowledge of human pathology, pathophysiology, or biochemistry while at the same time respecting the health and safety of the human subject.

Instrumentation, Imaging and Data Analysis

Basic Technology

Monitoring the amount of a radiolabeled drug or dosage form requires the use of appropriate instruments. These are used in the various stages of an experiment or study. Once the drug of interest or dosage form has been radiolabeled, it is necessary to assess the amount and integrity of the product. For reasons of safety, legality and propriety it is essential to have an accurate measure of the amount of the radioactivity to be administered and the amount retained as a standard source of reference. Standard radiation detectors are used for drug delivery research, and it is essential that strict quality assurance testing be performed on such instrumentation.

Once administered, the radiolabeled compound will be monitored in vivo over a period of time using appropriate imaging equipment. The main instruments used for this purpose are gamma cameras, which may be used for planar static and dynamic imaging and for single photon computed tomography (SPECT) imaging with single photon emitting radionuclides and positron emission tomography (PET) cameras for

imaging positron emitters. Such instruments can be found in widespread clinical use, although gamma cameras are more common than PET cameras. National and international quality standards for both imaging cameras and computer systems have been adopted. Image data are acquired, stored, and displayed using digital computers. Such systems also provide a means for the quantification of data. The use of camera-computer systems for monitoring and quantification of drug delivery, biodistribution, and pharmacokinetics places additional requirements on the quality control measures that need to be put in place.

Data Acquisition Formats

As used in the diagnostic imaging clinical setting, several data acquisition formats can be used for scintigraphic drug delivery research: static acquisition, dynamic acquisition, multiple energy acquisition, gated acquisition, and tomographic acquisition. Each is outlined in more detail in the sections that follow.

Static Acquisition

A static study represents the distribution of the radiopharmaceutical at a set time after administration; it is used when pharmacokinetics and transit are relatively slow. Static images are recorded in a set matrix over a predefined time. Typically, a static planar view would take approximately 30-60 seconds (imaging time is dose dependent) to obtain sufficient data for quantification. (A clinical bone scan view could take approximately 4 to 5 minutes to acquire.) In any case, statistically adequate counts are required. In a series of static views recorded over a long period, for example, up to 48 hours may be used to determine transit, release and distribution of orally administered drugs.

Dynamic Acquisition

A dynamic study would usually be carried out where the kinetics of the administered material is relatively rapid and all the required data can be collected in one image session. The subject would be continuously imaged over the period of the study and, therefore, has to remain still. Typical studies include bolus IV injections, hepatic uptake, renal excretion or swallowing an oral dose form. In a dynamic study, a series of consecutive frames of a given matrix size are acquired over the total length of the study.

Acquisition parameters for dynamic study:

Matrix size,
Frame time (seconds), and the
Total number of frames.

The data of regional uptake or clearance can be expressed in the form of activity time curves generated from a set of regions of interest defined on the dynamic images.

Multiple-Energy Acquisition

It is possible to simultaneously collect gamma rays from compounds radiolabeled with different radionuclides. Multiple energy windows can be used to filter out the different energies and images of specific gamma energies recorded. The most common form of this study is the dual radionuclide study where two specific components of a drug delivery system or compound may be monitored. In dual radionuclide, or dual isotope, studies the counts from two energy windows are simultaneously acquired into separate matrices of the same size. In this way, it is possible to record the images of two radiopharmaceuticals simultaneously

administered to a patient. The images can be digitally compared or subtracted pixel-by-pixel, using the computer to highlight differences. Examples of some dual radionuclide studies include:

- Simultaneous monitoring of a drug and a delivery device,
- An oral dose form and a dietary component (liquid or solid meal),
- Liquid and solid phases of an oral formulation, and
- A targeting moiety and a cytotoxic moiety.

Gated Acquisition

In this type of data acquisition, a trigger pulse taken from a physiological signal such as an electrocardiogram (ECG or EKG) waveform or respiratory gate initiates acquisition. In nuclear medicine, the most common study of this type is the cardiac gated study. Synchronization of data recording is obtained from ECG electrodes. Data are used to calculate the left ventricular ejection fraction, which is of value in monitoring the effects of drugs used in cardiology.

Tomographic Acquisition

Single photon emission computed tomography (SPECT) may be performed using a dedicated scanner, or using a rotating gamma camera system, to display the data as slices taken at chosen planes through the subject or as three-dimensional images.[14] The term SPECT signifies the detection of only one gamma photon at any time to produce an image data point. This is distinctly different from positron emission tomography (PET) in which two photon events arising from a positron emitting radionuclide are necessary for an accepted event. Most SPECT imaging is performed with a single or dual head gamma camera mounted on a gantry to facilitate circular rotation of the detector 360° around the patient. Data are acquired as a series of dynamic planar matrix views, typically 64 views in a 128 × 128 matrix.

The main advantages of SPECT are:

- An increase in the image contrast
- The visualization of data slice by slice
- The possibility of additional filtering of the image data to extract additional information
- The production of three-dimensional (3-D) display

A rigorous system of quality control and calibration is necessary for SPECT imaging. The assessment of the image acquisition and reconstruction parameters may be undertaken using phantoms (test objects).[15] The main variables affecting the collection of image data using rotating gamma cameras are listed below:

- Size of the image matrix,
- Number of angular increments for data collection,
- 180°or 360° rotation,
- Choice of collimator,
- Increment acquisition time, and
- Detector uniformity correction.

Image Reconstruction and Display

A computer program reconstructs SPECT images, using the same mathematical process (filtered back projection) as that used in x-ray computed tomography. The data in the planar views are projected onto the image matrix in the tomographic image plane, this process being repeated for all angles around the patient. To remove unwanted contributions from the reconstructed image, each data point has an associated negative part projected onto the

image. The main image processing variables are given below:

- Choice of image pre-filter,
- Choice of reconstruction filter,
- Attenuation correction (although not generally a routine part of SPECT, this is needed for accurate quantification),
- Scatter correction, and
- Slice orientation.

Images may be displayed as orthogonal, axial, coronal and sagittal slices, or as oblique cuts through any chosen plane. The three-dimensional pixel is known as a voxel. Once in this form, the data may be processed and viewed as a three-dimensional image using volume rendering computer software.[16] SPECT provides the most accurate means for the quantification of volume and the absolute quantification of organ uptake of single photon tracers. Volumes may be measured if the pixel size and slice thickness are known.

Data Analysis

One of the most powerful features of scintigraphic imaging is the ability to quantify the image data. Each pixel in the image represents the number of detected gamma rays from that area of the subject. It is therefore possible to quantify regional uptake over specific portions of the image. This is achieved by defining a region of interest (ROI) in the computer image. In this way, the uptake may be expressed as a percentage of the administered dose or directly in MBq.

The following factors need to be considered when quantifying data from scintigraphic images. The main points are given below:

The time and duration of image acquisition. The counts obtained from the images are dependent on the elapsed time between administration and imaging and the duration of data collection. The count rate is measured directly from the images.

Background subtraction. It is necessary to subtract a constant value from every pixel in the ROI to account for background levels of radioactivity,

Decay correction. It is necessary to account for radioactive decay with time, especially if a study is performed over a significant period when compared to the physical-half life of the radionuclide.

Gamma ray attenuation. Gamma rays are attenuated at depth in the patient due to interactions with overlying tissues. If the attenuation coefficient is measured using a transmission source, an appropriate correction can be made. Calculation of the geometric mean of the count rates in paired anterior and posterior planar views is often used to provide a more accurate measurement of quantification from planar images. This may be given by the following equation:

$$Cc = \sqrt{[Ca \times Cp]}$$

Where: Cc = corrected count rate.
Ca = anterior count rate.
Cp = posterior count rate.

Activity time curves: By defining regions of interest (ROIs) on a dynamic image series recorded over a period of time, the count rates may be used to generate radioactivity vs. time curves. Provided that the patient is imaged in the same anatomical position, the count rate obtained from the ROI in each frame of the series can be used

as a data point on the activity-time curve. This technique can be used to measure the rates of uptake, dissolution, spread and relative function. Once the curves have been generated, a number of pharmacokinetic parameters can be measured, for example gradients and areas under the curve.

PET Imaging

Positron emission tomography (PET) has emerged as one of the most powerful tools for understanding basic metabolic function in man. The ability of PET to image molecular pathways and molecular interactions in vivo has led to its increased use in both biological research and medical diagnosis. Positron emission tomography uses radionuclides that decay by the emission of positrons. Positrons are positively charged electrons (i.e., antimatter) that are short-lived and travel only a short distance of 1–2 mm before interacting with their matter counterpart (i.e., electron), resulting in the production of two annihilation photons. These 511 keV photons are emitted in directions 180° opposed to each other. Diagonally opposed detectors and a coincidence network are used to accept the two photons produced from one annihilation event. Imaging is usually performed on a dedicated PET scanner comprising a circular array of detectors and looking much like a CT scanner.

PET scanners have higher spatial resolution than SPECT systems. Transmission sources are used to correct for the attenuation of gamma photons in the patient, thus increasing the accuracy of data quantification. Positron emitting radionuclides generally have very short physical half-lives (2–109 minutes). A cyclotron located in close proximity to the PET scanner is therefore required for the production of these radionuclides. Rapid, automated labeling techniques also are required for radiopharmaceutical production, thus adding to the expense of the technique.[17]

A standard gamma camera may be used for detecting single 511 keV gamma rays from positron emitters provided high-energy collimation is used.[18] However, because of the thin crystals in standard cameras, the sensitivity of detection is low and image quality is poor. The development of dual-headed gamma camera systems with coincidence detection capabilities for 511keV photons will increase the availability of this technology to a larger number of centers.[19]

One of the main advantages of PET is the range of biologically active molecules that may be studied. Organic molecules may contain positron-emitting radionuclides like carbon-11 (^{11}C), nitrogen-13 (^{13}N), oxygen-15 (^{15}O) and fluorine-18 (^{18}F). Therefore, PET imaging offers a wide scope of possibilities for use by the pharmaceutical industry. From a radiochemistry standpoint, the main disadvantage in the use of positron-emitting radionuclides is that they generally have short physical half-lives, which reduces the time available for radiolabeling and manufacturing of the material under test; therefore, local access to a cyclotron is required for production. Because of the short physical half-lives of PET radioisotopes, PET radiochemistry must be performed rapidly. Chemical separation from the target material and radiolabeling of the test compound is usually performed using remote automated techniques. This is obviously a critical stage when considering a PET study since radiolabeling a novel drug or compound with a positron emitter requires careful preparation and planning.

Quality Assurance

All aspects of pharmaceutical research require careful calibration, validation, and documentation. This philosophy should include all aspects of instrumentation and computer equipment, including both hardware and software. Equipment calibration procedures should be performed at regular intervals to demonstrate consistent performance. A quality assurance program must incorporate written procedures for equipment calibration and records of the physical measurements made should be kept for audit purposes. Gamma camera calibration procedures using phantoms and test objects are well described in the literature.[20-23] Parameters such as detector uniformity, sensitivity, count rate performance, spatial resolution, and spatial distortion should all be measured. In addition, it is essential that regular checks should be made to ensure the safe operation of equipment.

SCINTIGRAPHIC DRUG DELIVERY STUDIES

The Gastrointestinal Tract

Oral dosage forms are comprised mainly of tablets, capsules, syrups, and suspensions. These are the most convenient forms of medications, and are therefore used in preference to any other route. Oral formulations are designed to be released in or to coat various portions of the gastrointestinal tract. Many formulations are designed to release after a set period of time or at specific site to maximize drug absorption and pharmacokinetic profile. For example, delivery of drug to the colon maximizes the local concentration of some therapeutic agents whilst minimizing exposure to the rest of the body, thus improving the efficacy of treatment and lessening potential side effects. This module is designed to illustrate how a gamma emitting radiopharmaceutical can be incorporated into an oral dose formulation and to demonstrate the role of gamma scintigraphy in the design of oral dosage forms. Examples of studies are provided from measuring esophageal transit to demonstrating specific targeted release in the colon.

Radiopharmaceuticals

In any scintigraphic study that is performed with the prime intention of measuring transit, it is essential that a non-absorbable radiopharmaceutical is used as the marker. In clinical studies of esophageal transit, the most frequently used radioactive bolus is 5-10 MBq of ^{99m}Tc-sulfur or tin colloid in 10-20 mL of water. It is also common practice to use either ^{99m}Tc colloid or pentetate (DTPA) incorporated into more solid marker such as a food product, for example, scrambled egg or mashed potato. It is possible to incorporate a range of different radiopharmaceuticals into tablets, capsules, and liquid suspensions. Capsules may be opened and a weighed amount of a radiolabeled powder added to the contents before closing. Tablets may be drilled using a small sterile drill bit. After filling with a radioactively labeled powder, the hole can be sealed with a suitable cement (e.g., an orthopedic adhesive) to give a smooth finish. Microscopic examination of drilled tablets should be carried out before release of the test items to ensure that the surface properties have not been adversely affected.

Radioactive powders for incorporation into formulations may be produced by a number of methods. Suitable powders include lactose, sucrose, and calcium phosphate. A small volume of an

aqueous solution containing the chosen radiopharmaceutical can be added directly to the powder, mixed to ensure dispersion, and allowed to dry, or dried by the application of heat. Labeling occurs through adsorption or absorption. Alternatively, the radiopharmaceutical can be dissolved in a solvent in which the powder itself is insoluble. The powder can be suspended in this radioactive solution, which is then evaporated to dryness. This method produces a uniform dispersion of radioactivity. Radiopharmaceuticals that can be incorporated by this method include:

- ^{99m}Tc-DTPA
- ^{99m}Tc-tin colloid
- ^{99m}Tc-sulfur colloid
- ^{111}In-DTPA

Radiolabeling can also be achieved by mixing finely dispersed ion-exchange resins with powders. ^{99m}Tc pertechnetate can be taken up onto an anionic resin such as Amberlite® IRA 416. In-111 indium as the chloride can be used with a cationic resin like Amberlite® IR120 (H).

If it is not possible to handle the intended formulation within the radiopharmacy, it may be preferable to add non-radioactive materials suitable for neutron activation during the manufacture of the formulation. Suitable nuclides of samarium and erbium have been employed. This requires that the formulation is placed in a nuclear reactor and bombarded with neutrons for an appropriate period. Caution is advised when undertaking such studies since the activation process may produce other radionuclides. A full analysis of the radionuclidic content of the activated formulation should be undertaken using gamma spectroscopy to ensure that no other radionuclides that may be inappropriate for human administration have been produced from other materials present in the formulation.

Esophageal Transit and Retention of Dosage Forms

The oral delivery of pharmaceutical dosage forms is still the preferred mode of drug administration. Immediate-release solid oral dosage forms are designed to pass through the esophagus and to disintegrate in the stomach for absorption in the small and/or large intestine. Solid oral dosage forms (tablets and capsules) are normally ingested with water or an appropriate fluid to ensure rapid delivery of the dosage form to the stomach. Once in the stomach, the dosage form disintegrates releasing the drug for dissolution and subsequent absorption.

The technique of gamma scintigraphy is currently the preferred method to monitor esophageal transit. Although x-ray studies may be used to monitor GI transit they involve significantly higher doses of ionizing radiation, which limits use in healthy subjects and the incorporation of dense contrast materials into the dosage form significantly alters the physiologic process under investigation. The scintigraphic study of esophageal transit was first introduced by Kazem, et al. (1972)[24] as a diagnostic test for examination of esophageal function. During scintigraphy the patient is asked to swallow a radioactive bolus (usually ^{99m}Tc) while positioned upright or supine with a gamma camera positioned over the anterior or posterior chest with its field of view extending from the cervical to the upper abdominal region. Typically, the camera is equipped with a low-energy all-purpose collimator, and is interfaced with a computer. During continuous image acquisition, the patient is asked to swallow the radioactive bolus and to subsequently swallow at 15–30 second

intervals with data acquisition for up to several minutes.

Quantification of esophageal transit was introduced by Tolin in 1979.[25] Data were acquired following the swallowing of a single radioactive bolus of water.

The count rate derived from regions of interest around the esophagus was used to determine esophageal transit rates with the use of the formula:

$$C_t = \frac{E_{max} - E_t}{E_{max}} \times 100\%$$

- Where, C_t = percent esophageal transit at time t,
- E_{max} is the maximal count rate in the esophagus, and
- E_t is the esophageal count rate at time t.

In adults less than 15 seconds is considered normal esophageal transit times for a liquid.[26]

Factors Affecting Esophageal Adhesion

Although younger patients tend to have a normal gastrointestinal history, esophageal injury has been reported in patients from age 3-90.[27] Elderly patients are particularly at risk because they have decreased mucous flow and low amplitude peristaltic propulsion. The incomplete ingestion of a pharmaceutical dosage form contributes to esophageal adhesion and potential injury. It is now generally accepted that esophageal transit is markedly affected by the posture of the subject and the amount of fluid used to swallow the dosage form.[28] If the tablets are taken without water, the risk of adhesion is greatly increased and the dosage form may remain in the lower esophagus until it disintegrates.[29] A potentially more dangerous situation is the ingestion of the dosage form at bedtime because esophageal transit rate, saliva production, and swallowing frequency all are reduced in the supine position. Recent reports document esophagitis with alendronate, an aminobisphosphonate, if the uncoated tablets are not taken with 6–8 oz of water or if ingested in the supine position.[29,30]

Formulation Effects

If the dosage form adheres in the esophagus, the active ingredient slowly dissolves creating a high drug concentration on the mucosal surface of the esophagus. Repeated or prolonged use of some drugs can result in serious iatrogenic injury. Scintigraphy permits quantification of the transit of any radiolabeled dosage form and it can identify the site of adhesion. The main factors that affect the esophageal adhesion of pharmaceutical dosage forms include:

- Shape of the dosage form
- Size of the dosage form
- Surface area
- Adhesive properties of the surface coating
- Density

The volume of fluid administered with the dosage form and the body position (erect or supine) has a significant affect on transit. It appears that administration of dosage forms with little to no water and swallowing in the supine position are the greatest factors that enhance the probability of adhesion. Large oval tablets taken with little or no water in the supine position have the greatest tendency to adhere to the esophagus.[31] Gelatin capsules are also noted to have slower transit to that of tablet formulations.[32] It is generally accepted that the formulations most prone to adhere are gelatin capsules. Film-coated tablets,

uncoated tablets and sugar-coated tablets show the least potential to adhere.[33,34]

The data on the formulation used in transit studies are often incomplete, and investigators vary the dosage form administration technique (volume of fluid and body position) to meet the intent of the study. In addition, patient age, disease state, and number of patients vary significantly. Therefore, if esophageal adhesion is an important parameter to monitor, it is recommended that esophageal transit studies be conducted using a specific formulation in a representative subject population.[26,35,36]

Summary of factors to be considered in study design:

- The population must be defined in terms of age, disease state and number of subjects;
- The study dosing conditions must be clearly defined and validated in terms of the specific fluid intake and body position requirements; and
- The criteria for adhesion must be defined, [i.e., period of time for delayed transit (e.g. > 20 seconds for adhesion)]

Esophageal Coating

The function of the esophagus is to deliver swallowed material to the stomach. The esophagus is very efficient at delivering virtually all of a swallowed bolus to the stomach, leaving very little residual coating. In certain clinical conditions, such as gastroesophageal reflux disease (GERD), it would be advantageous to coat the esophageal mucosa and protect it from damage caused by refluxed gastric acid and enzymes. To study the effectiveness of oral liquid esophageal coating formulations, scintigraphic imaging may be used to quantify esophageal mucosa and clearance of the radiopharmaceutical over time.

Typical Study Outline

Validation of the radiolabeled formulation should be undertaken to demonstrate that the tracer is appropriately associated with the formulation.

^{99m}Tc is added to each of the unit-dose syringes in a volume to deliver 2 MBq ^{99m}Tc in each dose of test product.

The radiolabeled unit doses are imaged by the scintillation camera in a "neck phantom" to obtain the total gamma counts contained in the dose. (A "neck phantom" is a device that approximates the attenuation of the gamma counts induced by the soft tissues of the neck, allowing more accurate correlation of in vivo and ex vivo counts.)

Subjects are positioned in front of the scintillation camera and the dose administered.

10-minute dynamic images (0.5 seconds/frame) are obtained to record the swallow and initial coating.

Immediately after the 10-minute dynamic study, the empty dosing unit is imaged in the neck phantom to determine the gamma counts remaining in the syringe.

The gamma count left in the syringe should be subtracted from the total gamma count to determine the gamma count of the actual dose given.

Static images are recorded every 15 minutes for one hour, and every 30 minutes for 5 hours.

The primary effectiveness measures include retained fraction, duration, and area under the curve (AUC) for retained fraction, for total esophagus and upper/mid/lower regions of the esophagus.

Radiolabeled Controlled-Release Formulations and Targeted Drug Delivery

Incorporation of a gamma-emitting radionuclide into a delivery device or formulation provides a simple and convenient method for observing the transit through, and residence within, the gastrointestinal tract. It is of particular use in determining the time and site of release of delayed release formulations. Unlike radiological assessment, it allows serial images to be taken without imposing an unacceptably high radiation burden to the subject, and therefore an accurate knowledge of site and release characteristics of the formulation can be gained. The main objective in selective GI drug delivery is to deliver the formulation through the stomach and small intestine, avoiding release or luminal degradation. In order to investigate the absorption of drugs at specific sites within the gut a number of engineering based capsules have been developed. Examples include InteliSite® and Enterion®. These can be labeled with a small amount of radioactivity to determine their exact position in the GI tract using the gamma camera. Once the correct position has been identified, the release mechanism is triggered by a radiofrequency signal. Assay of drug levels in blood can be used to determine bioavailability.[37a]

The most important variables are transit times (crucial to the success of timed release delivery systems) and the pH profile (determining the breakdown of the protective polyacrylic resins that constitute the 'enteric' coating of certain preparations).

Gastric Emptying

After ingestion and esophageal transit, the dosage form enters the stomach. Residence time within the stomach is dependent on both the physical characteristics of the dosage form, and on the presence or absence of food. Scintigraphy may be used to demonstrate the disintegration and emptying of immediate release formulations. By taking a series of anterior and posterior planar views, the rate of disintegration and emptying of a labeled formulation may be quantified. Regions of interest can be defined around the stomach and a geometric mean (refer to previous section on Instrumentation, Imaging, and Data Analysis) applied to the count rates to plot a clearance curve.

The stomach is able to discriminate between liquids, digestible solids, and inert indigestible solids. Hence, the gastric emptying time of a substance is largely determined by its physical form. It is well established that liquids empty faster than solids,[37] with the later being selectively retained until ground down to a suitable size in the antrum. Upon exiting the stomach, the dosage form enters the small intestine, where there is a dramatic rise in pH consequent to bicarbonate secretion from the duodenal mucosa and pancreas. In contrast to the stomach, the transit time through the post-prandial human small intestine appears to be relatively constant and unaffected by the dosage form (solutions, pellets, or large capsules) or stomach contents,[38] with a value of 3-4 hours in the post-prandial state.

Colonic Delivery

After dosing on an empty stomach, a formulation should reach the proximal colon approximately 4-5 hours after ingestion (Note: this time excludes gastric emptying). Indeed, several small studies have shown this to be the case using a large (25 × 9 mm) pressure sensitive radiotelemetry delivery system and 0.5-1.8 mm diameter radiolabeled resin pellets,[39] capsules of differing densities and volumes,[40] and 4 × 4

mm non-disintegrating tablets.[41] These preliminary studies suggest that delivery to the proximal colon can be achieved reasonably reliably and predictably provided meal patterns are standardized, the dosage form is taken in the fasting state, and thereafter a meal is ingested to encourage uniform passage to the colon.

The colon merits special consideration because transit within this region is highly variable both between individuals and within the same individual.[42] In terms of distal colonic disease, such as left-sided colitis or diverticulosis, delivery of formulation to the distal colon would be preferential, whereas if systemic delivery of a drug over a prolonged period were required, then release and retention within the proximal or right side of the colon may be more desirable, as discussed below.

Colonic Physiology and Drug Absorption

It is clear that the colon is not a single homogeneously functioning organ, but is better thought of as being composed of two distinct units, the proximal and distal colon. These two regions are embryologically distinct, the former being derived from the midgut and latter from the hindgut. It is therefore no surprise that each serves different functions, and indeed certain features of the proximal colon suggest that it may be better suited to drug absorption compared to the distal colon.

In recent years, the most commonly used method for assessing whole gut transit has involved the ingestion of radiopaque markers with subsequent radiographs of either the stool or the abdomen.[43] Using the time to excrete 80% of the administered pellets as a reflection of whole gut transit time, studies have shown that the mean whole gut transit time usually lies between 33 and 83 hours.[43-46] There are several problems with the radiopaque marker studies:

They impose a significant radiation burden, especially if they involve sequential abdominal x-rays;

Accurate localization of pellets may be a problem, particularly in the case of a sagging transverse colon; and

Radiographically dense markers may be treated differently due to chyme and propagated at a different rate compared to native material.

Radionuclide imaging has the advantages of low radiation exposure, the opportunity for sequential imaging without additional radiation burden, and allows more accurate quantitative analysis. The use of radioisotope and gamma scintigraphic imaging for assessing colonic transit in humans was first reported in 1986.[47]

In order to create a more physiological situation, bolus delivery of material has been achieved by packaging particulates (0.5-1.8 mm diameter pellets) within a gelatin capsule coated with a pH sensitive polymer. This prevents release until arrival in the distal ileum or proximal colon.[48] Studies using radiolabeled-Amberlite resin (0.5-1.8 mm), report ascending colon transit times of 12-13 hours.[48,49] Colonic transit times however appear to be shorter for larger objects,[39,48] with 80% of capsules reaching the splenic flexure within 10 hours of entering the colon in one study.[40] Thus, to achieve site-specific delivery to the left-sided colon, a 15-hour delayed release system would seem appropriate.

The proximal colon has a primary role in resorbing sodium and water before delivering the processed, dehydrated stool distally. Perfusion studies in the human colon have demonstrated larger net water and electrolyte movement in the proximal

than the distal colon,[50] with absorption most rapid in the cecum and decreasing progressively in the transverse colon, descending colon and rectum.[51,52] Electrical potential difference measurements support these perfusion studies, showing the distal colon to be more impermeable to sodium than the proximal colon as evidenced by the larger negative potential differences established distally.[53,54]

Enema Spreading

It is difficult to determine the degree of retrograde spreading of an enema formulation. However, scintigraphy can provide a simple indication of the rate of spread and retention of this form of medication. It is possible to radiolabel formulations including effervescent foams using ^{99m}Tc or ^{111}In. Providing the appropriate validation steps have been undertaken to ensure that the radiolabel is non-absorbable and well mixed with the formulation, scintigraphic images can be used to quantify the distribution and retention over time.[54a]

Imaging and Presentation of Data

Indium-111 is a radionuclide particularly well suited for whole gut, or colonic transit studies, chiefly because its half-life (67.5 hours) is similar to whole gut transit time and, therefore, good quality images are obtained while minimizing the absorbed radiation dose. In the chemical form of ^{111}In-chloride, it is easily adsorbed onto ion exchange resin to form a non-absorbable marker, ensuring compartmentalization within the gastrointestinal tract after ingestion.

High-quality images of the delivery and subsequent release of formulations in the colon may be obtained by adding as little as 1 MBq of ^{111}In-labeled Amberlite resin to the dosage form. Thirty-second anterior and posterior images are then taken at 30-minute intervals throughout the study periods. Release and subsequent spread of the labeled Amberlite resin allow an image of the colon to be constructed and used to establish the exact site of release of marker probes within the distal gut. Concomitant plasma and urine samples then allow an estimate of absorption that can be correlated to the site of release.

In addition to information on the site of release of formulation, an indication of the spread or dispersion of material can also be obtained. However, the addition of labeled Amberlite resin within the delivery vehicle presents an opportunity to estimate the degree of dispersion of released formulation. In studies of colonic absorption, the colon can be divided into eight regions of interest in a modification of the method described by Krevsky[47] (Figure 1). Counting the number of regions containing at least 10% of the total activity delivered by the vehicle at a set time point after the release of formulation can provide a semi-quantitative index of dispersion.

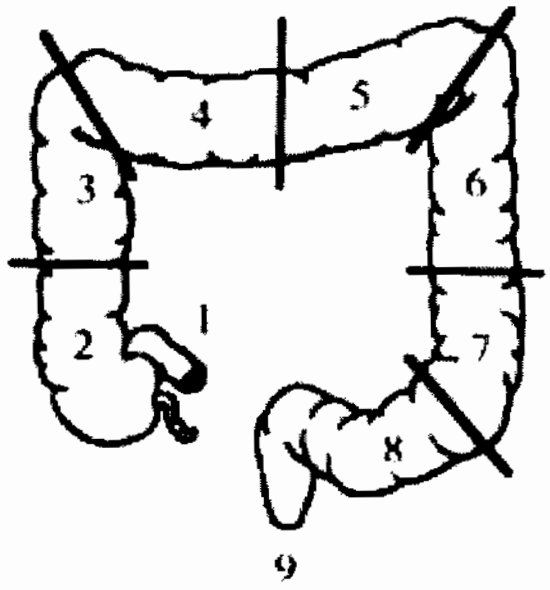

Figure 1. Colonic regions of interest. The ascending, transverse, and descending colonic regions may be defined by bisecting the hepatic and splenic flexures, and the junction between the descending and rectosigmoid colon.

Value of Studies in Formulation Design

In addition to providing data on gastrointestinal physiology, gamma scintigraphy can provide important data for the pharmaceutical industry on the formulation of their drugs. They provide tools for assessing the following in both normal and patient groups:

- Swallowing of tablet and capsule formulations;
- Measurement of disintegration of immediate release formulations;
- Measurement of gastric emptying and gastro-esophageal reflux;
- Demonstrating release of enteric coated formulations;
- Measurement of GI transit times;
- Measurement of the effect of formulation size on GI delivery, which may affect the timing of colonic targeting;
- Visualization of targeted release or delivery of a formulation to the colon, and assessment of the effects of time of dosing (morning or night-time) on delivery; and
- Investigating regional permeability within the colon, which has implications for bioavailability and the plasma profile of the drug;
- Studying the dispersion characteristics of oral formulations that are likely to influence drug absorption;
- Quantification of the residence of material within the colon in the equilibrium state and therefore assess the likely exposure of different regions of the colon to the formulation; and

- Quantification of site of delivery, spreading, and retention of enema preparations.

Case Study of Radiolabeled Solid Oral Dose Formulations

The oral delivery of pharmaceutical dosage forms is very important area of study. Tablets and capsules have been radiolabeled for the study of oesophageal transit and release, with the aim of ensuring rapid swallowing of potentially erosive drugs. Specific examples of formulations are given in the case study below.

Radiolabeling

Technetium-99m sodium pertechnetate was obtained at a concentration of 500MBq/ml by elution of a molybdenum-99/technetium-99m generator [Elumatic III, CIS (UK) Ltd]. Sterile ^{99m}Tc-sodium pertechnetate was added to approximately 150mg Amberlite ion exchange resin IRA 416 (Cl), particle size 0.3-1.2mm (BDH Laboratories Lot 0293140K). The mixture was then dried in a glass beaker using a hot air dryer. As an alternative, ^{99m}Tc sodium pertechnetate may be converted into the chelate ^{99m}Tc pentetate (DTPA) using a standard commercial kit (Mallinckrodt Medical Ltd) at high specific activity (approximately 500 MBq per mL). This material may also be dried onto powdered lactose to yield the modified dried fill to a final predetermined activity that ensures that each unit contains the required activity (i.e., 3 MBq ^{99m}Tc) at the scheduled time of dosing.

Capsules

Capsules were placed upright in a perspex support and opened. Using a microspatula, approximately 5mg of the contents was removed and 5 mg ^{99m}Tc-labeled Amberlite resin was added and the shell was firmly capped and the capsule inverted 6 times to mix the contents. The units were weighed prior to and after radiolabeling. The activity of ^{99m}Tc was calculated in order to give a radioactive dose of 3MBq per unit, accounting for the time delay between radiolabeling and administration. This resulted in an absorbed radiation dose to subjects of 0.15 mSv.

Tablets

The tablets were clamped and drilled at one edge using a 1.5-mm diameter drill bit sterilized with alcohol. Radiolabeled resin was tamped into the interior of the tablet using a modified Eppendorf pipette tip to facilitate loading of the resin in the fine drill hole. The units were finally sealed with a small amount of sterile bone cement. The tablets were visually inspected to ensure that surfaces were smooth and free from cracks due to the drilling process.

Product Evaluation

Stability of labeling and the effect of incorporating the radiopharmaceutical on the disintegration properties of the formulation may be evaluated *in vitro* at the investigation site prior to the clinical study. *In-vitro* studies undertaken in standard dissolution apparatus may be used to demonstrate that the release of radioactivity into solution coincides with visual observations of tablet dispersion, thus validating the scintigraphic procedure. These studies form part of the quality assurance process for manufacturing the formulations. Validation of the radiolabeling and imaging methodology is an essential component of scintigraphic studies.

In the case study presented here, the radiolabeled formulations were assayed immediately after radiolabeling the units and

immediately prior to administration using an ionization chamber dose calibrator. Dynamic images were recorded with a single headed gamma camera having a 40cm diameter field of view and fitted with a low energy collimator. Images were recorded over a period of 10 minutes and processed to give a condensed image display to show the position of the formulation in the esophagus with time (Figure 2).

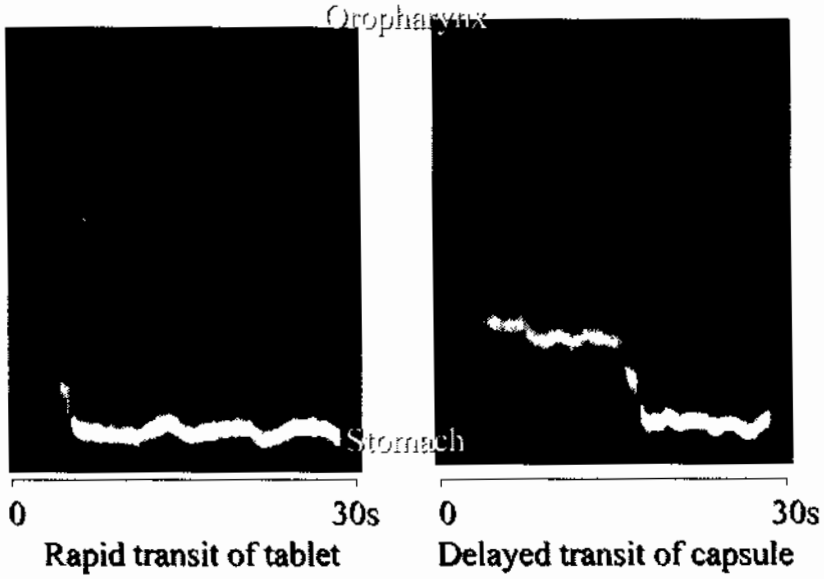

Figure 2. This figure shows a condensed image display of esophageal transit of labeled tablet and capsule formulations. Top of the trace represents oropharynx and bottom the trace is the stomach. Time is along the X-axis.

The Eye

The topical route is the most commonly utilized method to administer a medication to the eye. It is adequate for the treatment of most external conditions of the eye and those of the anterior segment, including the iris and ciliary muscle. It would be expected that the introduction of drug directly to the conjunctival sac should localize the drug effect, facilitate drug entry that is hard to achieve by systemic delivery and avoid first-pass metabolism. In practice, topical application frequently fails to establish a therapeutic drug level for a desired length of time within the target ocular tissues and fluids. Reasons include rapid precorneal clearance, the highly selective absorptive properties of the corneal barrier, unproductive drug loss via the conjunctival route and the difficulty that many people, particularly the elderly, have in administering eye drops to the eye. Typically, less than 5% of the instilled dose reaches the aqueous humor.[55,56]

In Vivo Methods for Assessment of Ocular Retention

Drug levels in the aqueous humor or other interior tissues in man are difficult to ascertain directly and a number of indirect methods have been developed to estimate

ocular bioavailability. These include tear sampling, the dye dilution test, pharmacodynamic evaluation, reflectance spectrophotofluorimetery, gamma probes, and lacrimal scintigraphy.

Tear sampling methods have been described to follow the precorneal kinetics of 0.3% ofloxacin and 0.3% tobramycin ophthalmic solutions in healthy volunteers.[57] The method involves the removal of a small volume of tear fluid from the eye. The process of sampling requires great care as the removal of volumes as small as 1µl, representing more than 10% of the basal tear volume, can disturb the dynamics of the tear film. Bach[58] utilized a dye dilution technique to evaluate the ocular residence times of hydroxypropylmethyl cellulose and polyvinyl alcohol solutions. Argyrol marker was applied to stain the corneal and conjunctival epithelium. Similar methods employing fluorescein/rose bengal are described in the literature. These techniques provide a limited ability to measure turnover. Reflectance fluorometry is a non-invasive technique first described by Maurice.[59] The technique has been used extensively to measure intra-ocular kinetics and the behavior of some drug vehicles, for example, the residence of gellan gums in the eye. The application is limited to transparent structures and linearity is restricted.

Use of radioactive markers detected by external gamma probes was first described in 1972.[60] The technique was used to measure the residence time of ophthalmic formulations labeled with ^{99m}Tc in the rabbit. An important limitation of this technique is that it is not possible to accurately resolve the distribution of the activity over the eye surface and drainage system. Lacrimal scintigraphy, using a radiolabeled probe and a gamma camera, sometimes fitted with a pinhole collimator is an attractive alternative technique. The technique was described[61] as a method to assess blockages of the nasolacrimal duct. In comparison to fluorometry, lacrimal scintigraphy provides a quantitative measure of the precorneal distribution and a simultaneous measurement of the proportion of the dose drained down the lacrimal duct.

Scintigraphic Studies

For human ophthalmic use, the isotope of choice is ^{99m}Tc in order to restrict dosimetry to the radiation-sensitive lens. The usual procedure involves the incorporation of a small amount of sterile fluid containing ^{99m}Tc at high concentration into the formulation. Deposition and clearance of the material is followed by serial measurements of the eye to determine the precorneal activity-time profile. The technique is associated with a low dosimetry, typically a 0.05-mSv effective dose, and therefore can be used on healthy male and female volunteers.

Radiolabeling of Ocular Formulations

Simple non-viscous aqueous solutions have the disadvantage that most of the instilled solution is lost within the first 15-30 seconds post-instillation due to rapid drainage[62]. In an attempt to prolong ocular contact times of drugs, a variety of viscosifying agents, including hydroxyethylcellulose (HEC), polyvinyl alcohol, sodium hyaluronate, gellan gum and carbomer, and other novel systems has been employed. The ocular pharmacokinetics of these formulations has been widely investigated using gamma scintigraphy.

Hydroxyethylcellulose

Cellulosic excipients can be conveniently labeled by direct addition of

^{99m}Tc-DTPA. Sodium pertechnetate is required at high concentration of around 2500 MBq in 1mL. Test materials are conveniently provided as sterile 5 mL ampoules or unopened material passed for human use. In a typical investigation, samples are radiolabeled on the day of the trial to give an activity of 1 MBq per dose at the time of administration. The labeled formulations should be used within three hours of preparation.

Polyvinyl Alcohol (PVA) Inserts

Aseptic production of inserts based on PVA containing pilocarpine nitrate and ^{99m}Tc-DTPA has been performed [63] using a highly soluble form of the polymer (PVA) with a median molecular weight of 98,000 and a 87-89 mol% degree of hydrolysis. The casting solutions were prepared at a concentration of 2.58% w/w to allow for dilution of the formulation to 2% w/w with the radioactive marker. A stainless steel hand-spreader was used to cast a PVA/Pilocarpine Nitrate film onto a Melinex backing strip. A film approximately 35 x 8 cm was cast and left to dry in a ventilation cupboard for approximately 1 hour, and cut into 25 mm^2 sections using a sharp scalpel. The inserts were then stored in a sterile petri dish until required.

Gellan Gum

Gellan gum is a multifunctional hydrocolloid that can be used to prepare structured liquids, sometimes referred to as "fluid gels." The gelling properties of gellan gums are related to the ability to form a double-helix structure in spite of the presence of substituents and side chains. Association between double helices is facilitated by ions and water molecules and a colloidal suspension environment of low ionic strength is relatively fluid. In the presence of the cations found in the normal tear film, interactions between the polymer helices lead to entanglement and a rapid rise in viscosity. To label gellan gum, the material is prepared as an over-strength solution (0.75% w/v). ^{99m}Tc-DTPA at an appropriate radioactive concentration is added to the gellan gum to give a final concentration of 0.6% w/v.

Mucus Glycoproteins

Mucins secreted by the periocular apparatus have an important function, and various studies have been proposed to follow the kinetics of mucins in the tear film. A novel method of radiolabeling mucus glycoproteins was described,[64] based on an established method for labeling antibodies. Disulphide bridges within the glycoprotein are cleaved by the use of the reductant 2-mercaptoethanol. Following a subsequent purification using a Sephadex column, labeling is performed via Sn^{++} reduction of the pertechnetate in the presence of an excess of a weak competing chelating ligand, methylene diphosphate (MDP). To label the material, mucus glycoprotein suspension was mixed with 2-mercaptoethanol solution and left to reduce for 30 minutes. The mucus glycoprotein sample was passed through the Sephadex G-5 column and the column washed with Phosphate Buffered Saline. The washings were combined and 0.5 mL of the sample, 5 mL saline, and 1 mL ^{99m}Tc-sodium pertechnetate (approximately 500 MBq) added to an MDP kit. The reconstituted kit was then left for 2 minutes. The radiolabeled mucus glycoprotein was combined with a sample of unlabeled mucus glycoprotein to produce a 0.5% mucus glycoprotein suspension.

Carbopols

Carboxyvinyl-based polymers including Carbopol 940 have proved to be extremely useful in the formulations of sustained release gels. A four to ten fold increase in the corneal and aqueous humor concentration of prednisolone acetate or prednisolone phosphate suspended in Carbopol 940 compared to the reference aqueous suspensions has been demonstrated[65]. Quantitative investigations have been limited by the propensity of carbopols to cause changes in erythrocyte rheology in the presence of electrolytes. The addition of 0.05% sodium fluorescein or 0.01% w/v disodium edetate caused a significant change in the carbomer formulations and subsequent rheologic changes in erythrocytes.[66] Other studies have shown that incorporation of ^{99m}Tc-sodium pertechnetate in isotonic saline at concentrations of 4%-10% v/v into carbomer gels caused visible viscosity changes in the vehicle. It is necessary to reduce the concentration of sodium added in the labeling procedure. ^{99m}Tc-sodium pertechnetate is extracted into an equal volume of n-butanone. The organic supernatant is then transferred to a clean vial and dried under a stream of warm dry air in a laminar flow cabinet. Finally, the residue is dissolved in 100-µl DTPA solution reconstituted with sterile water. This modified low sodium ^{99m}Tc-DTPA adduct has been used for radiolabeling carbomer gel[67]. A comparison of the apparent viscosities of GelTears®, (a proprietary product based on Carbopol 940) labeled with ^{99m}Tc-DTPA in isotonic sodium chloride solution, low sodium ^{99m}Tc-DTPA and the unlabeled product is shown in Figure 3.

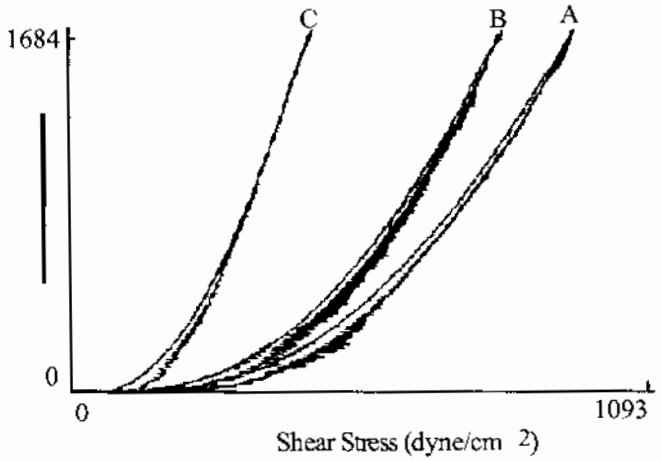

Figure 3. The effect of sodium ion on flow curves of GelTears (Data from Wilson 1998[13]). A: GelTears, h' = 1.93 cps; B: GelTears with low sodium ^{99m}Tc-DTPA, h' = 1.77 cps and C: GelTears with isotonic sodium ^{99m}Tc-DTPA, h' = 0.72 cps, where h' is apparent viscosity at shear rate 1684 s^{-1}.

Emulsions

Emulsions are defined as a mixture of two immiscible liquids, one of which is dispersed uniformly in small droplets throughout the other. The dispersed liquid is termed the internal or discontinuous phase, and the dispersed medium is termed the external or continuous phase. The system is stabilized by the addition of an emulsifying agent. Emulsions, particularly those based on castor oil, are used as vehicles in ocular delivery systems. Radiolabels can be incorporated into either the aqueous phase or the oil phase. The aqueous phase presents few problems, since the majority of ^{99m}Tc radiopharmaceuticals exists in the form of hydrophilic, polar molecules, and therefore provides models for water-soluble drugs. For the study of emulsion formulations containing lipophilic drugs, alternative models must be considered.[68] Some lipophilic radiopharmaceuticals have been examined,[69] to assess their suitability as drug models in an emulsion comprising castor oil and water. Radiopharmaceuticals investigated were ^{111}In-oxyquinoline (oxine) (Indium-Oxine, Amersham Health); ^{99m}Tc-exametazime (Ceretec®, Amersham Health); ^{99m}Tc-sestamibi (Cardiolite®, DuPont); and three other ^{99m}Tc-hexakis isonitrile derivatives (*t*-butyl, *n*-butyl and *cyclo*hexyl) synthesized in the laboratory. Rates and extents of partitioning in an emulsified system comprising castor oil and water were assessed. All compounds showed a high degree of lipophilicity. Active compounds introduced into the castor oil phase generally remained associated with the oil although Ceretec® did show some losses of pertechnetate into the aqueous phase. Indium oxine demonstrated a high affinity for the oil phase, but in the presence of serum albumin 5.0% in the aqueous phase, some loss from the oil phase did occur. None of the technetium compounds behaved differently in the presence or absence of serum proteins. Active compounds introduced into the aqueous phase migrated at different rates into the castor oil. Ceretec® demonstrated the fastest rate, but it was unstable with ^{99m}Tc-pertechnetate detectable on HPLC analysis.

All compounds studied have potential as models for lipophilic drug formulations. The use of Ceretec® is limited by instability problems to studies of short duration (<30 minutes). The use of ^{111}In-indium oxine may be limited in situations where formulations come into contact with high concentrations of protein in solution. This is probably caused by a disassociation of the indium from the oxine in the presence of other ligands on the protein molecule with higher affinity constants for indium[70]. Isonitriles appear highly stable chemically, and remain associated with the oil phase for long periods (>2 hours). *t*-Butyl and methoxy*iso*butyl isonitriles both have an established use in myocardial imaging and have therefore been evaluated toxicologically. Other isonitrile compounds have similar properties, but they have not been tested in human subjects. Isonitriles offer great potential as lipophilic drug models but toxicological evaluation of some derivatives is necessary.

Particulate Suspensions

These can be simulated using commercially available particulate radiopharmaceuticals. One problem is that particle size and charge have significant effects on ocular clearance, and a useful alternative material in this respect is micronized carbon. This material has several advantages:

- It can be heat sterilized;
- It is available as a particulate with a mean size of around 5 μm
- It is inert
- It adsorbs ^{99m}Tc-DTPA with great avidity.

In the labeling process, sterile carbon (~50 mg) is mixed thoroughly using a sterile spatula with ^{99m}Tc-DTPA solution (~10 µL) and dried. The radiolabeled carbon is then added to the formulation.

Non-Aqueous Vehicle Systems

Perfluorocarbon (PFC) compounds have been used as a vitreous replacement during retinal surgery for the repair of giant retinal tears and the manipulation of intraocular foreign bodies such as dislocated intraocular lenses. For delivery of drugs to the eye, they offer certain advantages over aqueous formulations. The high surface tension of water (72.75 dynes cm^{-1} at 25° C) does not permit drop size to be reduced much below 25 µL. With suitable modification of an applicator tip, drops of 5 - 8 µL of perfluorodecalin can easily be dispensed since the surface tension is only 19.3 dynes cm^{-1}.

One problem with these materials is that they are inert: they are extremely poor solvents for both hydrophilic and hydrophobic drugs, which therefore must be incorporated as a suspension. Activated carbon, with ^{99m}Tc-DTPA adsorbed, provides a suitable model in this system for drug-delivery studies.

Imaging Procedure

For the test, the subject is encouraged to arrive early to allow sufficient time to acclimatize to the procedure. The subject is seated in front of a gamma camera fitted with a 3 mm aperture pinhole collimator and the head supported by an ophthalmic table positioning the eye 5 cm from the collimator. A suitable volume, usually less than 25 µL of the radiolabeled formulation, is then placed in the lower fornix according to a randomized allocation schedule. Precise time of the completed instillation of the test formulations is recorded. Clearance is normally monitored for 8 minutes using dynamic imaging (96 × 5 second frames) followed by a series of static images over a period of 30 minutes to 2 hours. Data are stored on optical disk for subsequent analysis. A suitable control such as saline should be used to allow for a clear interpretation of the data. Subjects sometimes stare into the collimator and the blinking pattern is abnormal. This is evident in the analysis, when a distinct "day 1" effect is noted. It is therefore important to ensure that the subject is comfortable and familiar with the technique. Contact lens wearers or subjects receiving ocular drug therapy or any other form of drug therapy on a regular basis would normally be excluded from this type of trial unless this was the object of the study.

Data Analysis

There are four analytical ways commonly used for the evaluation of the dosage forms:

- Measure the time necessary for eliminating 50% (T_{50}) or 63.2% (MRT) of the dose administered;
- Measure the proportion of the administered dose remaining after a given time;
- Calculate the values of the rate constants for the elimination of the drug in the fast and slow phases of the clearance profile.
- Calculate the values of AUC (areas under the % administered dose remaining vs. time curves).

The stored data are recalled and the dynamic sequence summed to give an image of the eye and the drainage apparatus. This image is then analyzed by creating five regions of interest: (1) cornea, (2) inner

canthus, (3) lacrimal duct, (4) whole eye, (5) background (see Figure 4). The total activity in the three anatomical ROIs (corneal, inner canthus, lacrimal duct) in the first frame is assumed 100% of the instilled dose, calculated from the first frame. The remaining activity in the ROI at each time point is calculated as a percentage of the initial activity. The count rates from the regions require correction for background and radioactive decay using a validated spreadsheet document. Percentage remaining in each ROI, as a function of time, can be plotted for each test solution in each subject. Data sets are then combined to produce mean plots (± sd) for each solution and for each region of interest.

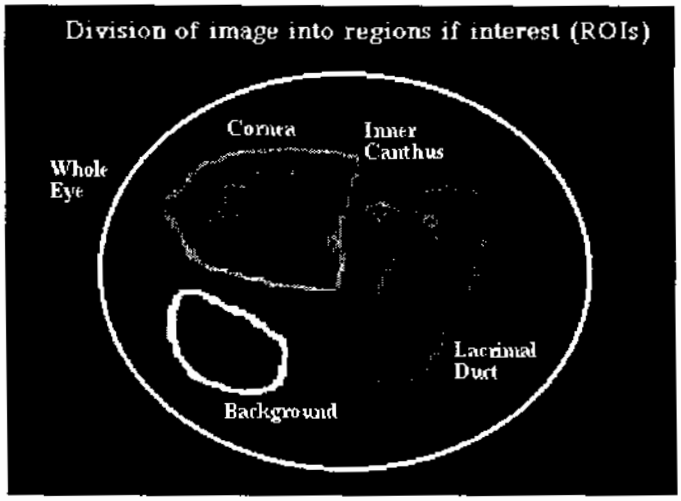

Figure 4. The generation of five main regions of interest (ROIs) in the eye: cornea, inner canthus, lacrimal duct, whole eye and background. This image was obtained in a human using a gamma camera and a pinhole collimator 5 mm in diameter, in a conical collimator about 30 cm from the camera surface and projecting on to a 30-cm diameter zone on the crystal. Matrix size of the image is 128 x 128.

Results

Mean activity-time data generated from dynamic study following administration of HEC solutions are shown in Table 2. As expected, the ocular residence of HEC formulations is related to the viscosity of the vehicles. A 0.1% HEC formulation is an almost non-viscous solution (4 cps), which would not be expected to increase retention. This is in agreement with the observed short precorneal residence time. The experimental data indicate that 0.5% HEC provides superior ophthalmic residence compared with the diluted HEC solutions or saline. This relatively inexpensive vehicle is therefore the viscosifier of choice in many ophthalmic formulations.

Table 2: **Mean Corneal Clearance Time of ^{99m}Tc-Labeled HEC or Saline Formulations in Humans (Data from Wilson[71])**

Formulation	Saline	0.1% HEC	0.3% HEC	0.5% HEC
AUC_{0-30} (%min)	258.8± 160.1	295.6+160.1	391.4+169.7	505.4+256.1
Mean Corneal Clearance Time	9.9+6.8	15.5+15.1	19.7+15.8	109.7+60.1

Studies with polyvinyl alcohol (PVA) inserts show significant increase in residence time over the same activity delivered as a conventional solution. Decrease in intraocular pressure in response to pilocarpine delivered by this route has also been shown to be significantly greater than with a saline formulation.[63] Gellan gum has been observed to remain in contact with the cornea at the site of application for up to 100 minutes.[72]

Clearance of nanoparticles has been shown to be multiphasic in nature with an initial rapid phase, followed by a slow phase. Emulsions have not been extensively studied, but they offer great potential as a delivery vehicle for lipophilic drugs. The use of non-aqueous vehicles produces extremely long retention times on the cornea, in excess of 2 hours for suspended drug models.[73] They represent a very promising approach to the delivery of drugs via the ocular route, but lack of knowledge about long-term toxicity remains a problem.

Summary

Over the past fifteen years, lacrimal scintigraphy has proved of great value in the assessment of ophthalmic drug delivery systems. Numerous published studies in man are to be found in the literature, and these are summarized in Table 3.

Table 3: **Ophthalmic Formulations Evaluated in Man Using Lacrimal Scintigraphy**

Formulations	Sources listed according to reference citations.
Methylcellulose	74
Polyvinyl alcohol	74,75; 76, 77, 63, 78,
Hydroxyethylcellulose	79, 72, 80,81
Hydroxypropylmethyl cellulose	75,76, 78,79
Xanthan gum	82
Gelrite®	72
Ointment	83
Hyaluronate	84, 78
Carbopol	67

Further developments in this field are limited by the availability of suitable markers, particularly (a) as lipophilic probes and (b) as small molecular weight peptides. The technique of conventional ophthalmic delivery is very ineffective and greater than 20% of patients cannot use dropper bottles effectively. The use of gamma scintigraphy to evaluate novel delivery devices and new polymers will no doubt afford researchers valuable insights into better ophthalmic therapy in the future.

The Lung

Radionuclide imaging has a well-established role in the development and evaluation of products for drug delivery to the respiratory tract. The principal application is in monitoring lung deposition of inhaled aerosols. Radiolabeling of the aerosol allows quantification of the distribution of the formulation. This is particularly relevant, since most inhaled formulations are administered for their topical actions. Nuclear medicine techniques are also applied to the monitoring of nasal formulations, mainly for determining deposition sites and measuring the rates of clearance of formulations from the nasal cavity. Administration of drugs directly into the lungs provides a high therapeutic index by allowing relatively low doses to be deposited to achieve high local concentrations at the sites of action. The main factor influencing lung drug deposition is particle size.[85] Particles with mass-median aerodynamic diameters greater than 5μm tend to deposit by impaction in the oropharynx and the large airways; particles between 0.5-μm and 5-μm diameter deposit in the lungs primarily by sedimentation, whereas deposition of the finer particles is largely dependent on diffusion. Radionuclide studies have played a major role in investigations of the effects of particle size and dosing procedures on lung deposition. Therapeutic aerosols are administered using a wide range of devices such as nebulizers, pressurized metered-dose inhalers and powder inhalers. Monitoring the deposition of radiolabeled aerosols provides a direct measure of the efficiency of such devices. Additionally, aerosol delivery systems have been developed specifically for the

administration of diagnostic aerosols for lung ventilation studies.[87]

As with the lungs, drugs are administered to the nasal cavity primarily for their local action, but there is increasing interest in this route for systemic delivery. Key factors readily amenable to investigation by gamma scintigraphy are the site and extent of drug deposition and the residence time in the nasal cavity.

Radiolabeling and Imaging

The radiolabeling of respiratory formulations is dependent on the nature of the product. Aqueous solutions for nebulization are often radiolabeled by the addition of ^{99m}Tc-DTPA. This tracer is absorbed from healthy lungs with a half-time of approximately 1 hour[88] and is rapidly cleared from the blood by the kidneys. The swallowed portion of the dose is excreted without absorption from the gastrointestinal tract, allowing differentiation between the fractions deposited in the lungs and the oropharynx. In situations where administration may be relatively prolonged, use of a less rapidly absorbed tracer such as ^{99m}Tc-labeled albumin may be advantageous. Additionally, if the rate of clearance is to be monitored, for example of a solution deposited in the nasal cavity, the tracer distribution should represent that of the constituent of interest.

Most pressurized metered-dose inhalers contain a suspension of the drug particles in the propellant. It is important that the distribution of the radiolabel should reflect that of the drug particles and that it is unaffected by the radiolabeling procedure. The most commonly adopted procedure involves extracting ^{99m}Tc-pertechnetate into an organic solvent and evaporating the solution to dryness in an empty aerosol canister. Using a cold transfer process, the contents of a canister of the formulation to be labeled are added to the technetium-containing canister and a metering valve attached.[89] Confirmation of satisfactory radiolabeling is achieved using a multistage impinger to compare the drug and radiolabel distributions from the labeled preparation with the drug distribution from a non-labeled canister of the same product. The radiolabeling of a propellant-soluble pressurized metered-dose inhaler involves the addition of a propellant soluble radiopharmaceutical, for example technetium ^{99m}Tc-exametazime, or direct labeling of the drug molecules. As with the suspension formulations, the drug and tracer distributions should be assessed *in vitro* in order to validate the labeling procedure.

Powder inhalers usually contain either pure drug or a blend of drug with a carrier, often lactose. The diameters of the carrier particles are generally much greater than the drug particles. In order to ensure the radiolabel is associated only with the drug particles, it is necessary to label the drug prior to blending with the carrier. The radiolabel, normally a compound of technetium-99m, is dissolved in a volatile solvent in which the drug particles are insoluble. The radiolabeled solution is added to the drug powder and the solvent evaporated. If necessary, the drug particles are disaggregated and blended with carrier before being loaded into the inhaler. The labeling procedure should be validated using impinger deposition studies.

Radiolabeling of Powder for Dry Powder Inhaler

A novel radiolabeling technique using Technegas™ has recently been reported for the study of inhaled budesonide.[90] Briefly, the procedure involves adding 100mg of the micronized budesonide onto the collection filter of

Technegas. Technegas was generated from sodium pertechnetate from the commercial generator. Technegas aerosol in argon was then passed through the micronized budesonide for labeling with the Technegas particles. The labeled budesonide was blended with 325M lactose (Drug concentration; 5%). Finally, 4 mg of this blend was filled into a capsule for use with the Aerolyser™.

In Vitro Validation of the Radiolabeling Method

Particle sizing experiments were performed to determine whether the radiolabeling method affected the size distribution of the drug, and to determine the similarity of the particle size distribution between the drug and Technegas particles. All sizing measurements were performing using a Multi-stage Liquid impinger (MSLI; Copley, Nottingham, UK), operated with an inhalation flow of 95L/min according to British Pharmacopoeia. MSLI comprised a throat, four impaction stages and a final filter. The profile of drug and activity was measured to ensure that the label followed the distribution of the drug.

The respirable fraction (with a diameter< 5.4μM) was defined as the percentage of the drug or Technegas particles recovered from stages 3 to the final filter, on their total recoveries. The capsule, device, throat and stages of the MSLI were washed with 50mL of methanol. The absorbance (243nm) of each of the washes was measured using an ultraviolet spectroscopic method. The concentration of budesonide was determined using the absorbance of a solution containing a known concentration of budesonide and the percentage of the deposition of budesonide on the each region was calculated to the total recovery of budesonide.[89]

For lung deposition studies, the distribution of the radiolabeled formulation should be imaged immediately following administration. Once in the lungs, weakly bound tracer is likely to redistribute or clear at a different rate from that of the drug. For planar gamma camera imaging, normally anterior and posterior images are recorded to include the lungs and stomach. Additionally, a lateral image is taken of the head and neck. Quantification requires correction for tissue attenuation of the counts detected.[90] Use of a multi-headed gamma camera may permit tomographic imaging before significant redistribution of the tracer has occurred. Tomographic imaging offers the potential of relating closely drug distribution to lung morphology.[91]

The distribution and clearance of formulations deposited in the nasal cavity can be monitored from lateral images of the head recorded at intervals, typically for up to 4 hours following administration. Images of the thorax taken soon after dosing may be helpful in confirming the lack of inhalation of nasal sprays into the lungs.

Aerosol Generation

Therapeutic aerosols may be generated by three types of devices: nebulizers, pressurized metered-dose inhalers, and dry powder inhalers.

Nebulizers

Nebulizers are used for converting aqueous solutions and suspensions into respirable droplets. They are of two basic types, jet nebulizers that rely on a stream of air to generate the aerosol, and ultrasonic nebulizers in which droplets are produced by the high frequency vibration of a piezoelectric crystal. Nebulizers are useful for the delivery of relatively high dose treatments to patients with severe chronic

obstructive pulmonary disease or asthma. They are also commonly used for the administration of antibiotics. Depending on the type of nebulizer used and the formulation, fill volumes range from about 2 mL to 6 mL, with nebulization taking 10 - 30 min. The aerosols delivered from nebulizers have mass median aerodynamic diameters (MMAD) of typically 1 μm to 5 μm; the actual values reported for a particular nebulizer-formulation combination are dependent on the measurement technique adopted. Differences in the design and operation of the nebulizers results in considerable variability in drug delivery, both in terms of particle size distributions and the rates of aerosol generation.[92]

Radionuclide imaging studies have been applied to the characterization of nebulizer performance. The deposition of tracer in the lungs tends to be low, generally less than 20% of the amount loaded into the device[93]. A study comparing eight different nebulizers to administer pentamidine aerosols to patients with AIDS showed that the average dose deposited in the lungs ranged from 1% to 5%.[94] Most of the drug remains concentrated within the device or deposited on the delivery tubes and baffles intended to filter out the larger droplets. Additionally, aerosol may be wasted by generation during the non-inspiratory phase of respiration. In general, nebulization occurs more rapidly using ultrasonic nebulizers than with jet nebulizers. The droplets generated, however, tend to be larger than those from the ultrasonic nebulizers, resulting in relatively more central lung deposition.

The physicochemical nature of the formulation also affects lung deposition. Chan and colleagues[95] using radiolabeled solutions showed that following inhalation of similar sized aerosols, hypotonic droplets deposited more peripherally than hypertonic droplets. The effect was more apparent at low droplet concentrations in the airways and was attributed to the tendency of hypotonic droplets to shrink while the hypertonic droplets grew by taking up moisture. Other formulation considerations include the surface tension of the liquid. The addition of up to 1% surfactant, for example, to facilitate nebulization of a suspension decreases surface tension and tends to increase the MMAD of the aerosol.[96]

Radionuclide imaging studies are useful in the assessment of administration techniques. It has been shown, for example, that about twice the amount of aerosol deposits in the lung when inhaled through the mouth compared with nasal breathing.[97,98] The distributions within the lungs were, however, the same for both oral and nasal inhalation. Such information is particularly relevant in the treatment of infants, since they inhale primarily via the nose even when their mouths are open.[98] Lung deposition data, provided by gamma scintigraphy, can be used to aid in the selection of the most appropriate system for delivery of a particular treatment. For example, the choice of nebulizer for the administration of pentamidine as a prophylaxis against *Pneumonocystis carinii* pneumonia in patients with AIDS has received considerable attention.[94,99] Nebulizers resulting in high peripheral lung deposition with relatively low oropharyngeal deposition provide the most effective therapy while minimizing the unpleasant side effects of the treatment. Another situation in which the choice of nebulizer may be important is the administration of gentamicin in cystic fibrosis. It has been demonstrated by Smaldone[100] that highest sputum levels are associated with more central lung deposition of the drug. In

general, drug delivery from nebulizers is poorly controlled. With the introduction of a wider range of inhaled drugs, it is becoming increasingly important to ensure more precise dosing. The ways in which nebulizers are used, for example, in terms of solution volume and airflow rate for each pharmaceutical formulation, need careful consideration. Gamma scintigraphic studies can aid in demonstrating the formulation is administered from the most suitable device to provide deposition appropriate to the condition being treated.

Pressurized Metered-Dose Inhalers

Pressurized metered-dose inhalers (MDI) have been available since the mid-1950s; by 1996, annual production was in excess of 400 million units. They are readily portable and provide a convenient means of dosing patients having a wide range of respiratory diseases. Approximately 80% of inhaled asthma therapy is delivered by MDI. Most metered-dose inhalers comprise a metal canister fitted with a metering valve and containing drug suspended or dissolved in chlorofluorocarbon (CFC) or hydro-fluoroalkane (HFA) propellants. The canister is fitted into a plastic actuator from which the dose is delivered. The majority of currently available MDIs contain a suspension of drug particles 1-2 μm diameter. Each canister contains typically 100–200 doses of between 20 μg and 5 mg per actuation. Drug delivery occurs over about 0.1 s; the spray velocity averaging about 10 m/s over the first 5 cm. This results in many patients having trouble using MDIs correctly. The main problem, which may affect half the users, is an inability to coordinate actuation of the inhaler with inspiration of the dose. Additionally, many patients cease inhaling in response to the propellant impacting on the throat. In order to improve drug delivery, MDIs are frequently used along with a spacer device attached to the actuator mouthpiece. Additionally, breath-actuated inhalers have also been developed in order to overcome the coordination problem. Initial studies[101,102] monitoring radiolabeled particulate deposition and changes in lung function in response to bronchodilators administered from MDIs indicated that for optimum drug delivery MDIs should be actuated early during a slow inspiration followed by breath holding. In a recent study, Farr and colleagues[103] used a microprocessor controlled MDI to deliver ^{99m}Tc-labeled salbutamol particles at three inspirational airflow rates, and during early and late stages of inhalation. Each administration was followed by breath holding for 5 seconds. The maximum average lung deposition of 19% was achieved when the inhaler was actuated at an airflow rate of about 90 L/min, early during inspiration. The corresponding values of 14% and 8% were obtained with airflow rates of 30 and 250 L/min, respectively. Lung deposition was reduced by delivery of the dose late during inhalation. The proportion of the lung dose depositing peripherally was not significantly affected by the dosing procedure. Oropharyngeal deposition was about 60% with low airflow at actuation and increased to around 70% with the highest airflow rates. For all the administration conditions, only about 1% of the dose was exhaled. These findings confirm that the inhaler should be actuated early during inspiration at a relatively slow airflow rate. Lung deposition from MDIs is influenced by the formulation. Radionuclide imaging studies have shown that for drug suspensions, the proportion of the dose depositing in the lungs tends to increase as the drug content per actuation decreases (Table 4).

Table 4: Lung Deposition from Metered-Dose Inhalers

Drug	Dose per actuation (μg)	Lung deposition (%)	Reference
Nacystelyn	2000	12	109
Budesonide	200	18	121
Salbutamol	100	19	103
Salbutamol	100	22	107
Salbutamol	100	24	108

This is probably due to the generation of a larger proportion of droplets containing more than one drug particle at higher drug concentrations. This will result in an increase in the average aerosol particle size and hence a reduction in lung deposition. Even with low drug concentrations, lung deposition is generally less than 20% of the dose from the canister, with approximately 65% impacting in the oropharynx and the remainder depositing in the actuator. With suspension MDI drug formulations, the minimum aerosol size is that of the drug particle. For a solution formulation, dispersion and evaporation of the propellant governs the droplet size distribution. Studies with a CFC propellant soluble radiolabeled tracer, technetium ^{99m}Tc-exametazime, resulted in lung depositions of about 40%.[104] Lung deposition was independent of the actuator orifice diameter, indicating that evaporation of the droplets was of overriding importance. This has been supported by a study by Harnor and colleagues[105] in which lung depositions of 51% and 65% were achieved by dosing with a propellant having relatively low and high vapor pressures, respectively. The propellant having the higher vapor pressure would be expected to evaporate more rapidly, resulting in smaller inhaled droplets. Thus, there is potential for significant improvements in lung deposition by modifications to the formulations. Due to the ozone-depleting effects of CFCs, their use is being phased out in MDIs and HFA propellants are replacing them. Differences in the physicochemical properties of the two types of propellants have necessitated modifications to device components and drug formulations. The main effect on drug delivery results from some drugs, formulated as suspensions in CFC-based propellants and others as solutions in the HFA-based propellants. Leach[106] measured lung depositions of 51% for a BDP-HFA formulation compared with 4% for a BDP-CFC product, with a corresponding reduction in oropharyngeal deposition to 29% from 85%. Although these lung deposition values may be overestimated due to the count rates not having been corrected for tissue attenuation, they confirm the improvement that can be achieved with solution MDIs. The study by Leach[106] included a comparison of the lung deposition of aerosol from a BDP-HFA solution MDI in asthmatic patients and healthy subjects. Similar values were found for both groups. This finding is in agreement with the data reported by Melchor and co-workers[107] for a salbutamol suspension MDI. The mean total lung deposition in the patients (18%) was not significantly different from that in healthy subjects. A greater proportion of the lung dose was deposited peripherally in the healthy subjects compared with patients (Figure 5), 44% compared with 30%. Even with trained, healthy subjects being dosed under close supervision, the proportions of the dose depositing in the lungs may vary by a factor

of two or more. For example, in six subjects inhaling salbutamol, the mean lung deposition was 24% with a range of 15%-37%.[108] In six subjects who were dosed with nacystelyn, the mean value was 12% with a range of 9%-16%.[109] With patients adopting poor inhalation techniques, the variability is much greater. For eight asthmatic patients inhaling salbutamol from an MDI, lung deposition ranged from less than 1% to 28%[110]. The inability of patients to coordinate well the actuation of the MDI with inhalation has been addressed by the introduction of breath-actuated inhalers. These devices are designed to depress the canister and deliver a dose automatically at a predetermined inspiratory airflow rate, of typically 20-30 L/min.[112,113] In a study using radiolabeled salbutamol, Newman[7] showed that patients unable to coordinate correctly had, on average, lung depositions of 7% using standard MDIs, which was less than half that achieved in patients using a good technique. In the same group of poor coordinators, lung deposition from a breath-actuated inhaler averaged 21%, comparable with that attained by good coordinators. Thus, by ensuring drug delivery at a relatively slow inspirational airflow, the canister should be actuated early during inspiration thereby aiding lung deposition.

Figure 5. Drug delivery from a pressurized metered-dose inhaler, showing more central lung deposition in (a) an asthmatic patient than in (b) a healthy subject.

An alternative approach to improving lung deposition in poor coordinators is to actuate the MDI into an aerosol holding chamber, a large volume spacer, from which the patient subsequently inhales. This eliminates the need for precise synchronization between actuation and inhalation. Such spacers typically have volumes of about 700 mL. The larger aerosol particles are removed by impaction on to the spacer walls, while the contained aerosol droplets evaporate, resulting in finer particles for inhalation. Much of the dose may be lost rapidly to the spacer walls due to the electrostatic attraction between the aerosol and the spacer[113]. Depending on the formulation, the spacer material and design, and the interval between actuation of the MDI and inhalation, the patient may receive a similar, greater, or lower dose to the lungs than from a correctly used MDI. On average, most poor coordinators would be expected to achieve an improved dose to the lungs following administration via a large volume spacer. Melchor and colleagues[107] investigated the effect of a large volume spacer on the deposition of ^{99m}Tc-labeled salbutamol form and MDI. The drug was administered to both healthy volunteers and asthmatic patients, trained in the correct use of the inhaler. Use of the spacer did not affect total lung deposition in either group. A greater proportion of the lung dose, however, deposited deeper in the lungs when administered via the spacer. The oropharyngeal dose in the patients was reduced from 50% to 6% by use of the spacer: deposition in the spacer being 45%. In contrast, Newman[112] found increased lung deposition, from 15% to 28%, of ^{99m}Tc-labeled flunisilide in healthy subjects dosed from an MDI via a 250-mL spacer. This was accompanied by a reduction in oropharyngeal deposition from 67% to 27%. Such studies serve to illustrate the variable effects of spacers, even when used under optimum conditions. Reduction in oropharyngeal deposition can be achieved

with a small spacer, having a volume of about 50 mL. Such spacers function as extensions to the actuator mouthpieces, rather than as holding chambers. They are effective for a wide range of MDI formulations. With nacystelyn 2 mg/actuation, oropharyngeal deposition was reduced by 85%[109]; with beclomethasone dipropionate 100 g/actuation, oropharyngeal deposition was reduced by 80%. With a propellant soluble drug, oropharyngeal deposition was reduced by 77%.[104] With the propellant soluble drug, lung deposition increased from 38% to 57%. Thus for patients who are good coordinators, or breath-operated inhaler users, a small volume spacer provides a convenient alternative to a larger spacer as a means of reducing oropharyngeal deposition.

Dry Powder Inhalers

Dry powder inhalers are of two types, those containing drug predispensed in individual doses in capsules or blisters and those having a reservoir of powder from which doses are metered in the devices. Pure drug powder of a particle size suitable for inhalation into the lungs tends to be auto adhesive resulting in poor flow properties. The powders used in inhalers, therefore, are either loose aggregates of pure drug or blends of the drug with a carrier material, often lactose, of larger particle size. Since it is usually the drug distribution that is of interest, radiolabeling of blends requires that the drug particles be labeled before mixing with the carrier. To obtain meaningful drug deposition data for commercial powder inhalers requires that the radiolabeled powders match those of the original formulations. Most powder inhalers rely on the patients' inspiration to withdraw the drug from the inhaler and to disperse the particles. Sufficient airflow must be generated through the device. Drug delivery from powder inhalers is further complicated by the different resistances of devices to airflow[114]. These factors have to be taken into account for both the in vitro and in vivo evaluations of powder inhalers. While an airflow rate of 60 L/min through a low resistance inhaler such as the Rotahaler® can be regarded as low; this airflow rate could not be achieved by healthy subjects using the high resistance Pulvinal® inhaler[115]. Gamma camera studies have been used extensively to assess factors, such as inhalation flow rate, that affect lung drug deposition from powder inhalers. A study of sodium cromoglycate delivery from capsules in the Spinhaler® had been undertaken by Newman.[116] Inhalation at 120 L/min with the head tilted back resulted in lung deposition of 13%, while the corresponding value for inhalation at 60 L/min was only 6%. Approximately one third of the dose remained associated with the device. In the same study, the effect of breath holding and head position were investigated. Lung deposition was not significantly improved by breath holding for 10 seconds. With the head held in the normal position, lung deposition increased to 17%. A relatively low lung deposition of 8% was reported for sodium cromoglycate from a capsule delivered using the Rotahaler®.[117] Total lung depositions of 12% and 11% of salbutamol following blend delivery from blisters were not significantly different in healthy subjects and asthmatic patients, respectively.[108] These values were significantly less than the corresponding values of 22% and 18% from a pressurized metered-dose inhaler. As with the MDI, the drug was deposited more centrally in the lungs of the asthmatic patients. Salbutamol lung deposition from a blend in the multidose Pulvinal® powder inhaler was 14% when inhaled with maximum effort, at in an airflow rate of 46 L/min.[115]

Much higher lung drug deposition values have been reported for the Turbohaler®, a multidose inhaler delivering pure drug. Most asthmatic patients can achieve a peak inspiratory airflow rate in excess of 60 L/min through this device.[118] Using gamma scintigraphy the lung depositions of budesonide and terbutaline sulphate inhaled at approximately 60 L/min were 28% and 27%, respectively, of the metered dose.[119] When inhaled at 35 L/min, the lung deposition of budesonide was reduced to 15%. This reduction in lung deposition was accompanied by an increase in oropharyngeal deposition from 58% to 67%. Similar lung deposition data have been obtained from studies monitoring drug absorption and excretion. Such studies require the administration of charcoal to block absorption of the drug deposited in the mouth and throat. A slightly lower lung deposition value of 21% was obtained by analysis of terbutaline excretion in the urine following inhalation for the Turbohaler®.[120] A comparison of budesonide lung deposition, by measuring blood concentrations of the drug inhaled from the Turbohaler® and a pressurized MDI, showed the Turbohaler® to be twice as efficient as the MDI.[121]

Gamma scintigraphy has been used extensively in the development and assessment of powder inhalers. The two factors having the greatest influence on lung deposition are inhaler design and inhalation flow rate. Improvements in the design of powder inhalers are leading to more efficient and reproducible drug delivery. At the same time, the inhalation route appears more attractive for the administration of controlled release formulations and of systemically acting drugs having poor bioavailability following administration via the gastrointestinal tract.

Particle Clearance from the Lungs

The fate of inhaled particles is particularly important for formulations intended to provide drug release over prolonged periods. Insoluble particles depositing in the bronchi and bronchioles are cleared from the lungs by mucociliary clearance. Depending on the depth of penetration into the lungs, retention times in the ciliated airways are likely to range from a few minutes to several hours, with negligible amounts being retained at 24 hours.[122] In contrast, insoluble particles depositing in the alveoli are retained for prolonged periods. Studies in healthy volunteers have shown that about 50% of 5 μm diameter particles, gently inhaled followed by breath holding, are retained in the lungs at 24 hours.[123] After inhaling particles 5 nm in diameter, there is very rapid clearance over the same period, indicating that almost the entire dose is deposited in the alveoli.[124]

Particles deposited in the alveoli are cleared by phagocytosis by alveolar macrophages and by translocation of the particles across the alveolar epithelium.[125] The alveolar macrophages migrate to the terminal bronchioles, with a clearance half-time of several months, from where they are removed by mucociliary action. The particles in the connective tissues are likely to be phagocytosed by interstitial macrophages and remain relatively immobile.

To provide sustained delivery of drug within the lungs in excess of a few hours requires the particles to be deposited in the alveoli, to avoid clearance by mucociliary action. Within the alveoli, the particles are likely to undergo phagocytosis, which may prevent effective delivery of the drug to its site of action. Studies using

radiolabeled tracers will be useful for determining the fate of such formulations.

Nasal Drug Delivery

The nose acts as a filter for removing particles from the inhaled air. Most of the particles deposit anteriorly in the nasal cavity, in the region free of cilia. Clearance from this region is relatively slow and occurs by traction of the mucus layer into the ciliated posterior two-thirds of the nasal cavity. In healthy subjects, ciliary action clears mucus into the nasopharynx at approximately 5 mm/min, resulting in a particulate residence time in the ciliated region of about 15 min.[126] Mucociliary function can be greatly affected by pathology; for example, the common cold can both increase and decrease nasal clearance.[127,128] Other conditions, such as cystic fibrosis, chronic sinusitis, polyposis, Sjøgren's syndrome and Karagener's syndrome have been shown to decrease particulate clearance from the nasal cavity.[129,130] Gamma scintigraphy has been extensively applied to the study of nasal mucociliary function. Drugs are administered into the nose for both topical action in the nasal cavity and systemic action. The nasal route of drug delivery is attractive, since administration is relatively easy, enzymatic drug degradation is low, and absorption is rapid. The permeability of the nasal mucosa to drugs, however, decreases with increasing molecular size. Additionally, the mucus layer provides a barrier between the drug and the underlying epithelium. Drug delivery can be enhanced by the application of formulations designed to prolong retention in the nasal cavity and the incorporation of penetration enhancers.[131]

It has been shown by gamma scintigraphy that nasal sprays deposit mainly in the non-ciliated anterior third of the nasal cavity. Clearance from the deposition site is predominantly via the inferior meatus and into the pharynx, with little spreading over the turbinates. The deposition pattern is little affected by the type of spray, being similar for mechanical pumps,[132] a pressurized metered-dose inhaler[133] and a powder insufflator.[134] Changes in spray cone angle of the pumps,[128] tilting of the device during dosing[133] and gentle or vigorous inhalation[135] had no significant effect on deposition. Clearance of non-viscous solutions of ^{99m}Tc-labeled human serum albumin followed a biphasic pattern with an overall half-time of typically 20-30 minutes.[132] None of the spray was detected in the lungs. Nasal drops, applied with the subjects supine followed by tilting of the head, resulted in extensive coverage of the walls of the nasal cavity.[135] Greater coverage was achieved by increasing the solution volume from 30 µl to 90 µl. This deposition in the ciliated regions enhanced the overall clearance rates compared with the solution applied by spray. Residence of a spray formulation in the nasal cavity can be prolonged by increasing the viscosity of the solution using hydroxypropyl methyl-cellulose.[136] Although the deposition patterns were the same for all the solutions investigated, the clearance rates decreased with increasing viscosity. For the solution with the highest kinematic viscosity studied, 430 mm^2s^{-1} at 20°C, the clearance half-time was 2.2 hours. Illum and colleagues[134] investigated albumin, starch, and DEAE-dextran microspheres as potential nasal drug delivery systems. The powders form bioadhesive gel-like structures when in contact with the mucus. The powders were labeled with ^{99m}Tc and shown to deposit anteriorly in the nasal cavity, with little reaching the turbinates. Slowest clearance, with a half-time of about 4 hours,

was obtained with the DEAE-dextran. The systemic activity of nasally administered drugs is dependent on the nature of the formulation and the mode of administration. Gamma scintigraphy provides a useful tool for monitoring the distributions of formulations in the nasal cavity and for investigating the effects of modifications designed to enhance retention.

Summary

There is increasing interest in the potential of the respiratory tract as a route of administration for drugs being developed as a result of advances in biotechnology.[137,138] Alongside the pharmaceutical developments, devices are being improved to deliver doses more accurately and more efficiently to their sites of action. Gamma scintigraphy has a well-established role in the assessment of inhalation products. There are likely to be increasing demands for such studies in this area of rapid scientific advancement.

Positron Emission Tomography for Drug Delivery Studies

Positron Emission Tomography (PET) uses short-lived positron emitting radionuclides bound to drugs and compounds for imaging drug metabolism within the body. PET imaging has provided basic physiological information that has aided the understanding of biochemical transformation and metabolism in the human body. The major clinical applications of PET are in oncology, coronary heart disease, and neurology, while the research applications include the in vivo mapping of receptor binding, pharmacokinetics and drug interaction in both animals and man. This form of imaging is regarded as a powerful research tool of value in both academic medicine and pharmaceutical industry.

Because positron-emitting radiopharmaceuticals are short lived, with half-lives of between a few minutes and several hours, a cyclotron is needed on-site to carry out all but a limited range of PET examinations. ^{18}F-fluoro-2-deoxy-D-glucose (FDG) is the main exception, which can be delivered to sites up to two hours traveling time from a cyclotron. This allows a subset of common PET examinations to be carried out for the purpose of clinical diagnosis on a routine basis at a clinical facility remote from the cyclotron.

When applied to the pharmaceutical sciences, PET imaging facilitates faster drug development through the production of metabolic images of the new drug's distribution within the body. The very nature of positron emitters that include elements such as oxygen, nitrogen and carbon, is more appropriate for the direct labeling of drug molecules than metals such a technetium or indium, which are more commonly used for gamma camera imaging. Radiolabeling in this way allows the amount of the drug in the target organ or tissue to be quantified with a high degree of accuracy, thus demonstrating specific molecular interactions in vivo leading to the improved development of new drugs.

PET Radiopharmaceuticals

A large number of positron emitting radionuclides are available. Most PET studies are carried out with the organic elements ^{11}C, ^{13}N, ^{15}O and ^{18}F, however several other radionuclides are available for use including ^{38}P, ^{73}Se, ^{75}Br and ^{76}Br. There are also some interesting generator systems available facilitating the short-term local production of ^{62}Cu, ^{68}Ga and ^{82}Ru. The physical data are given below in Table 5.

Table 5: Physical Data on the More Commonly Used Positron Emitters

Nuclide	**T 1/2**	**Mode of decay (%)**	**Nuclear reaction**
^{11}C	20.4 min	β^+ (99.8) EC (0.20)	$^{14}N(p,\alpha)\,^{11}C$
^{13}N	9.7 min	β^+(100)	$^{12}C(d,n)\,^{13}N$
^{15}O	2.1 min	β^+ (99.9) EC (0.1)	$^{14}N(d,n)\,^{15}O$
^{18}F	109.6 min	β^+ (97) EC (3)	$^{20}Ne(d,\alpha)\,^{18}F$
^{38}K	7.6 min	β^+ (100)	$^{35}Cl(\alpha,n)\,^{38}K$
^{73}Se	7.1 h	β^+ (65) EC (35)	$^{75}As(p,3n)\,^{73}Se$
^{75}Br	1.6 min	β^+ (75.5) EC (24.5)	$^{76}Se(p,2n)\,^{75}Br$
^{76}Br	16.1min	β^+ (57) EC (43)	$^{76}As(p,n)\,^{76}Br$

Table 6: Physical Data on the More Commonly Used Positron Emitters

Nuclide	T 1/2	Mode of decay (%)	Nuclear reaction
Generator Systems			
^{62}Zn	9.2 h	β^+ (93) EC (7)	$^{63}Cu(p,2n)^{62}Zn$
↓			
^{62}Cu	9.7 min	β^+(98) EC(2)	
^{68}Ge	271d	EC (100)	$^{69}Ga(p,2n)^{68}Ge$
↓			
^{68}Ga	68.3 min	β^+ (90) EC (10)	
^{82}Sr	25d	EC (100)	$Mo(p,spall)^{82}Sr$
↓			
^{82}Rb	1.3min	β^+ (96) EC (4)	

Due to the short half-lives of the useful positron emitters, rapid methods for chemical separation and subsequent radiolabeling are required. The target may be solid, liquid, or gaseous. In each case, an appropriate method of extraction, purification, and radiolabeling must be used. Further details can be obtained from Stocklin and Pike.[139] Because of high activity and high-energy gamma emission (0.511 MeV), radiolabeling and dispensing operations are often undertaken in densely shielded isolators (hot cell) using automated PLC-controlled apparatus. (The half and tenth values of thicknesses of lead for 0.511 MeV photons are 6 mm and 17 mm, respectively). The use of computer controlled robotic apparatus is increasing. For the more commonly used clinical materials such as ^{18}FDG, a modular "black box" synthesis unit is commonly incorporated into the mini cyclotron facility for routine batch production. Such systems are usually computer controlled.

Examples of experimental and clinical applications of PET radiopharmaceuticals are given in Table 6 below. (Specific published studies are given subsequently.)

Table 7: Examples of Experimental and Clinical Applications

PET Radiopharmaceutical	Examples of Medical Use
^{15}O-oxygen	oxygen metabolism
^{15}O-carbon monoxide	blood volume
^{15}O-carbon dioxide	blood flow
^{15}O-water	blood flow
^{13}N-ammonia	blood flow
^{18}F-FDG	glucose metabolism
^{18}F-FMISO	hypoxic cell tracer
^{11}C-SCH23390	dopamine D_1 receptor
^{11}C-flumazenil	benzodiazepine receptor

Radiosynthesis of positron emitting radiopharmaceuticals often involves the following:

- Irradiation of the appropriate target material and holder,
- The production of high initial radioactivity,
- Efficient extraction of precursor from target,
- Automated or remote controlled method of production,
- Appropriate radiolabeling chemistry,
- Radiologically safe dispensing facility,
- Compliance with cGMPs, and

Quality management.

For research use in pharmaceutical development, the radiolabeling of the drug molecule or entity is a substantial component of the study. As with all nuclear medicine imaging investigations, the radiolabeling must be validated before undertaking the imaging study. This will incorporate the evaluation of aspects of stability, functionality and safety of the final formulation. Procedures for manufacture and toxicity of radiotracers intended for pre-phase I PET studies in cancer patients have been produced by Aboagye and associates.[140]

Image Formation

The nature of the physical detection process and image formation has been previously described in this lesson. Following positron emission the particle readily combines with an orbital electron producing an annihilation reaction. According to the famous equation $e=mc^2$, mass and energy are conserved. The result is the production of two gamma photons each having energy of 511keV and traveling in opposite direction (180° opposed). The gamma photons are detected by opposing detectors and accepted by a coincidence detection unit. The acceptance of a true positron event is registered on the image as a detected count. The detection of two coincidence events provides images of higher spatial resolution than those produced by single photon emission tomography with gamma cameras. In modern systems, a CT scanner is incorporated into the PET scanner to provide an anatomical image. In this way, a functional anatomical map (FAM) may be obtained to aid localization of the sites of uptake and clinical interpretation of the

investigation. In the case of experimental studies, the additional radiation dose from the CT study may be unacceptable for use in normal healthy subjects.

Quantification of uptake is generally expressed in terms of a Standard Uptake Value (SUV), Where, SUV= activity concentration / (injected dose/body weight)

For the determination of drug biodistribution in experimental small animal models, high-resolution microPET systems have been constructed.[141] Such systems have increased spatial resolution compared with clinical systems and can provide valuable pre clinical information on drug metabolism.[142]

Clinical Utility

The most widely used PET radiopharmaceutical is ^{18}FDG, which behaves in the body like glucose. ^{18}FDG is selectively taken up by the heart, brain and most tumors due to increased glucose metabolism. Clinical utilization is therefore most widely applicable in the fields of oncology. ^{18}FDG is of particular clinical value in staging tumors such as lung, breast, colon and lymphoma.

FDG also has a valuable role in neurology and cardiology[143,144]. Clinical studies are now routinely undertaken and a general consensus for appropriate clinical use is now emerging.[153] It is important however to establish normal deviations from abnormal when used for clinical evaluation of patients.[146] Because of the ease of production and relatively long physical half-life of ^{18}F, there has been much effort in the radiolabeling of other materials such as peptides and amino acids with this radionuclide.[147,148] In addition to staging and diagnosis of disease, PET imaging is playing an increasing role in monitoring treatment and recurrence of cancer during chemotherapy.[149-151] It follows that this method is of great importance in assessing the use of new treatments such as in chemotherapeutic clinical trials.

Pharmaceutical Studies

The physiological basis of radionuclide techniques coupled with the ability for quantification of data gives nuclear medicine techniques a unique edge over other imaging modalities for the visualization of drug distribution in man. Studies may be undertaken to assess drug delivery using a model drug or a radiolabeled carrier rather than the active drug itself. However, an important attribute of PET studies is that the drug itself may be radiolabeled by incorporation of a suitable radionuclide. The nature and stability of the labeling will depend upon whether the study is intended to examine release, deposition, retention or dispersion, or is being used to monitor the effects of a physiological process such as the effect on gastrointestinal transit. In PET imaging, the incorporation of radioactive atoms of carbon, nitrogen, oxygen, or fluorine offers a mechanism for radiolabeling the drug or receptor molecules.

An example of the potential of PET imaging can be seen in the study of first pass liver function of drug. It is widely considered that the hepatic first pass metabolism prohibits oral delivery of many drug molecules and compounds. Such processes can be quantified in vivo to determine the hepatic extraction fraction and washout, thus aiding the accurate modeling of drug distribution.[152]

Neuropharmacology

Some of the early classical studies of metabolic function have been undertaken in the study of the brain. These studies have not only demonstrated basic physiological

and metabolic processes, but have been instrumental in the understanding of receptor mechanisms.[153-156] PET studies have provided powerful images of neuroreceptor binding and this modality is still regarded as one of the main imaging techniques of value to the pharmaceutical industry today. The in vivo study of such processes has been an integral part of drug design for the treatment of neurological conditions such as Parkinson's and Alzheimer's disease and in the treatment of drug addiction.[157,158] Sophisticated studies are now playing an important role in the development of drugs for the treatment of anxiety and depression. For example, 5-hydroxytryptamine [1A] (5-HT[1A]) receptors have been implicated in the pathophysiology of such conditions and the dose occupancy studies have been undertaken in normal subjects after labeling 5-HT[1A] antagonist with ^{11}C.[159]

Cancer Chemotherapy

The importance of cancer as a major killer is reflected in the growing development of new drugs for cancer chemotherapy. Both SPECT and PET imaging are playing an increasing role in the development of novel targeted drug delivery systems and the understanding of tumor physiology including T-cell activation.[160,161] Imaging studies can be used to demonstrate "proof of principle." Radiolabeling the drug molecule with a PET tracer can allow visualization of drug localization and metabolism in both target and non-target sites[162]. Amino acids are known to be the building blocks of proteins. It has long been understood that they serve essential functions in normal cellular growth. They also provide a useful indication of tumor growth. Since the introduction of PET, most studies of amino acid metabolism have been undertaken using ^{11}C as the radionuclide.[163] More recently ^{18}F has been employed, its longer physical half-life being more suited to the time course of protein synthesis.[146]

Future Development

The use of PET studies is steadily growing. Throughout the United States, Europe, and Asia the number of PET and cyclotron installations is growing. Imaging will continue to play an increasing role in the evaluation of new therapeutic strategies such as gene therapy delivery.[164,165] The development of new drugs will benefit greatly from an improved understanding of drug metabolism, receptor occupancy, and washout. Two good examples are the use of PET imaging in diabetic medicine for the determination of the insulin receptor function and the study of diabetes-induced changes in the serotonin neuroreceptor system.[166,167] Such experimental studies aid in the understanding of drug design, accelerating the development of new treatments.

A powerful feature of nuclear molecular imaging is the evaluation of drug delivery systems as performed in those patient groups for whom the treatment is intended. Physiological processes, and hence biodistribution, can be significantly altered in the disease state. Imaging studies in man are of far greater relevance than animal studies, and nuclear medicine techniques probably represent the only methodology currently available for the quantification of drug release and pharmacokinetics in specific patient groups. Such data are now routinely used as part of the documentary evidence that may be submitted to regulatory authorities such as the U.S. Food and Drug Administration. Practicality, cost and radiation dose are all major factors

CONCLUSION

The information included here is intended to provide an overview of the use of nuclear medicine techniques in the development of drug delivery systems. It does not include applications of tracer technology in studies of drug metabolism. A specific benefit of the technology is that it allows real-time investigation into the in vivo behavior of drug delivery systems coupled with the ability to relate formulation location and behavior to other parameters such as drug plasma levels or pharmacological effects. The value of the technique is now recognized by the fact that regulatory bodies accept and recognize the data generated in this way as an essential part of drug registration documentation.

REFERENCES

1. Bailar JC, Neddleman J, Berney BB, McGinnis JM. *Assessing Risks to Health—Methodological Approaches*. Ashburn Press; London, Connecticut: 1993.
2. Belzer R. The use of risk assessment and benefit cost analysis in U.S. risk-management decision making. In: *Risk Assessment: International Conference. 1992*; Session 5-9:420- 442.
3. Raven A. *Clinical Trials, An Introduction*. 2nd ed. Radcliffe Medical Press; New York: 1993.
4. *ICH Harmonized Tripartite Guideline for Good Clinical Practice*. ICH Secretariat; 1997.
5. World Medical Assembly. *Declaration of Helsinki*. Helsinki, Finland, 1964, and amended Tokyo, Japan, 1975; Venice, Italy, 1983; and Hong Kong, 1989.
6. Publications of the International Commission on Radiological Protection (ICRP) 1990 Number 60—Recommendations of the ICRP.
7. Hardy JG, Jasuja AK, Frier M, Perkins AC. A small volume spacer for use with a breath-operated pressurised metered dose inhaler. *Int J Pharm*. 1996;142:129-133.
8. Wilson CG, Bakhshaee M, Stevens HNE, Perkins AC, Frier M, Blackshaw EP, Binns JS. Evaluation of gastro-resistant pulsed release delivery system (Pulsincap) in humans. *Drug Deliv*. 1997;4:201-206.
9. Parr AF, Jay M. Radiolabeling of intact dosage forms by neutron activation: effects on in vitro performance. *Pharm Res*. 1987;4:526-528.
10. Awang MB, Hardy JG, Davis SS, Wilding IR, Parry SJ. Radiolabeling of pharmaceutical dosage forms by neutron activation of samarium-152. *J Labelled Comp Radiopharm*. 1993;33:941-948.
11. Digenis GA, Sandefer EP, Page RC, Doll WJ. Gamma scintigraphy: an evolving technology in pharmaceutical formulation development Part 1. *Pharm Sci Technol Today*. 1998;1:100-108.
12. IPSM Report No. 44. *An introduction to emission computed tomography*. Institute of Physical Sciences in Medicine; York: 1985.
13. AAPM Report No. 22. *Rotating scintillation camera SPECT acceptance testing and quality control*. American Association of Physicists in Medicine; New York: 1987.
14. Wallis JW and Miller TR. Three-dimensional display in nuclear medicine and radiology. *J Nucl Med*. 1991;32:534-546.
15. Stoklin G and Pike VW. *Radiopharmaceuticals for Positron Emission Tomography. Methodological Aspects*. Kluwer Academic Publishers; Dordrecht: 1993

16. Van Lingen A, Huijgens PC, Visser FC, Ossenkoppele GJ, Hoekstra OS, Martens HJM, Huitink H, Green MV and Teule GJJ. Performance characteristics of a 511-keV collimator for imaging positron emitters with a standard gamma camera. *Eur J Nucl Med.* 1992;19:315-321.
17. Jarritt PH and Acton PD. PET imaging using gamma camera systems. *Nucl Med Commun.* 1996;17:758-766.
18. NEMA NU1. *Performance of scintillation cameras.* National Electrical Manufacturers' Association; Washington: 1986.
19. IPSM Report No 65. *Quality Standards in Nuclear Medicine.* Institute of Physical Sciences in Medicine; York: 1992
20. IPSM Report No. 66. 1992 *Quality control of gamma cameras and associated computer systems.* Institute of Physical Sciences in Medicine; York: 1992.
21. British Standard BS6609. *Methods of test for Anger type gamma cameras.* British Standards Institution: London: 1992.
22. Kazem I. A new scintigraphic technique for the study of the esophagus. *Am J Roentgenol Radium Ther Nucl Med.* 1972;115:681-688.
23. Tolin RD, Malmud LS, Reilley J, Fisher RS. Esophageal scintigraphy to quantitate esophageal transit (quantitation of esophageal transit). *Gastroenterology.* 1979;76:1402-1408.
24. Blackwell JN, Hannan, WJ, Adam RD, Heading, RC. Radionuclide transit studies in the detection of esophageal dysmotility. *Gut.* 1983;24:421-426.
25. Kikendall JW, Friedman AC, Oyewole MA, et al. Pill-induced esophageal injury: Case reports and review of the medical literature. *Dig Dis Sci.* 1983;28:174-182.
26. Channer KS, Roberts CJC. The effect of posture at the time of administration on the efficacy of the hypnotic zopiclone. *Br J Clin Pharmacol.* 1984;18:879-886.
27. Hey H, Jorgensen F, Sorensen K, Hasselbalch H, Wamberg T. Esophageal transit of six commonly used tablets and capsules. *Br J Med.* 1982;285:1717-1719.
28. De Groen PC, Lubbe DF, Hirsch LJ, Daifotis A, Stephenson W, Freedholm D, Pryor-Tillotson S, Seleznick MJ, Pinkas H, Wang KK. Esophagitis associated with the use of alendronate. *N Engl J Med.* 1996;335:1016-1021.
29. Wamberg T, Jorgensen F, Hasselbalch H, Hey H. The rejudgment of the esophageal transfer of tablets and capsules. *Arch Pharm Chem Sci Ed.* 1983;11:24-31.
30. Perkins AC, Wilson CG, Blackshaw PE, Vincent RM. Dansereau RJ, Juhlin KD, Bekker, Spiller RC. Impaired esophageal transit of capsule versus tablet formulations in the elderly. *Gut.* 1994;35:1363-1367.
31. Marvola M, Vahervuo K, Sothmann A, Marttila E, Rajaniemi M. Development of a method for study of the tendency of drug products to adherence to the esophagus. *J Pharm Sci.* 1982;71:975-977.
32. Swisher DA, Sendelbeck SL, Fara JW. Adherence of various oral dosage forms to the esophagus. *Int J Pharma.* 1984;22:219-228
33. Klein HA, Wald A. Normal variation in radionuclide esophageal transit studies, *Eur J Nucl Med.* 1987;13:115-120.
34. Marshall JB, Bodnarchuk G, and Singh A. Supine and upright radionuclide esophageal transit before and after

treatment for achalasia. *Clin Nucl Med.* 1994;19:683-686.
35. Wilding I, Hist P, Connor A. Development of a new engineering-based capsule for human drug absorption studies. *Pharm Sci Technol Today.* 2000;3:385-392.
36. Minami H, McCallum RW. The physiology and pathophysiology of gastric emptying in humans. *Gastroenterology.* 1984;86:1592-1610.
37. Davis SS, Hardy JG, Fara JW. Transit of pharmaceutical dosage forms through the small intestine. *Gut.* 1986;27:886-92.
38. Hardy JG, Wilson CG, Wood E. Drug delivery to the proximal colon. *J Pharm Pharmacol.*1985;37:874-877.
39. Parker G, Wilson CG, Hardy JG. The effect of capsule size and density on transit through the proximal colon. *J Pharm Pharmacol.* 1988;40:376-377.
40. Hardy JG, Davis SS, Khosla R, Robertson CS. Gastrointestinal transit of small tablets in patients with ulcerative colitis. *Int J Pharmacol.* 1988;48:79-82.
41. Degen LP, Phillips SF. Variability of gastrointestinal transit in healthy women and men. *Gut.* 1996;39:299-305.
42. Hinton JM, Lennard-Jones JE, Young AC. A new method for studying *Gut* transit times using radiopaque markers. *Gut.* 1969;10:842-847.
43. Burkitt DP, Walker ARP, Painter NS. Effect of dietary fibre on stools and transit times, and its role in the causation of disease. *Lancet.* 1972(ii):1408-1412.
44. Cummings JH, Jenkins DJA, Wiggins HS. Measurement of the mean transit time of dietary residue through the human *Gut. Gut.* 1976;17:210-218.
45. Cummings JH, Branch W, Jenkins DJA, Southgate DAT, Houston H, James WPT. Colonic response to dietary fibre from carrot, cabbage, apple, bran, and guar gum. *Lancet.* 1978(i):5-9.
46. Krevsky B, Malmud LS, D'Ercole F, Maurer AH, Fisher RS. Colonic transit scintigraphy: a physiological approach to the quantitative measurement of colonic transit in humans. *Gastroenterology.* 1986;91:1102-1112.
47. Proano M, Camilleri M, Phillips SF, Brown ML, Thomforde GM. Transit of solids through the human colon: regional quantification in the unprepared bowel. *Am J Physiol.* 1990;258:G856-862.
48. Barrow L, Steed KP, Spiller RC, Watts PJ, Melia CD, Davies MC, Wilson CG. Scintigraphic demonstration of accelerated proximal colonic transit by lactulose and its modification by gelling agents. *Gastroenterology.* 1992;103: 1167-1173.
49. Levitan R, Fordtran JS, Burrows BA, Ingelfinger FJ. Water and salt absorption in the human colon. *J Clin Invest.* 1962;4:1754-1759.
50. Devroede G, Phillips SF. Failure of the human rectum to absorb electrolytes and water. *Gut.* 1970;11:438-442.
51. Devroede GJ, Phillips SF, Code CF, Lind JF. Regional differences in rates of insorption of sodium and water from the human large intestine. *Can J Physiol Pharmacol.* 1971;49: 1023-1029.
52. Rask-Masden J. Simultaneous measurement of electrical polarization and electrolyte transport by the entire normal and inflamed human colon during in vivo perfusion. *Scand J Gastroenterol.* 1973;8:327-36.
53. Davis GR, Santa Ana CA, Morawski SG, Fordtran JS. Permeability characteristics of human jejunum, ileum, proximal colon and distal colon: results of potential difference measurements

and unidirectional fluxes. *Gastroenterology*. 1982;83:844-850.
54. 54a. Jay M, Digenis GA, Foster TS, Antonow DR. Retrograde spreading of hydrocortisone enema in inflammatory bowel disease. *Dig Dis Sci*. 1986;31:139-144.Wood E, Wilson CG, Hardy JG. The spreading of foam and solution enemas. *Int J Pharmacol*. 1985;25:191-197.
55. Jarvinen T, Jarvinen K. Prodrugs for improved ocular drug delivery. *Adv Drug Deliv Rev*. 1996;19(2):203-224.
56. Limberg M, Bugge C. Tear concentrations of topically applied ciprofloxacin. *Cornea*. 1994;13(6):496-499.
57. Tang-Liu DDS, Schwob DL, Usansky JI, Gordon YJ. Comparative tear concentrations over time of ofloxacin and tobramycin in human eyes. *Clin Pharmacol Ther*. 1994;55(3):284-292.
58. Bach FC, Adam JB, McWhirter HC, Johnson JE. Ocular retention of artificial tear solutions. *Ann Ophthalmol*. 1972;4:116-119.
59. Maurice DM, Srinivas SP. Use of fluorometry in assessing the efficacy of a cation-sensitive gel as an ophthalmic vehicle: Comparison with scintigraphy. *J Pharm Sci*. 1992;81(7):615-619.
60. Chrai SS, Patton AM, Mehta A, Robinson JR. Lacrimal and instilled fluid dynamics in rabbit eyes. *J Pharm Sci*. 1973;62:1112-1121.
61. Rossomondo RM, Carlton WH, Trueblood JH, Thomas, RP. A new method of evaluating lacrimal drainage. *Arch Ophthalmol*. 1972;88: 523-525.
62. Shell J. Therapeutic Review: Pharmacokinetics of topically applied of ophthalmic drugs. *Surv Ophthalmol*. 1982;26:207-218
63. Greaves JL, Wilson CG, Birmingham AT, Richardson MC, Bentley PH. Scintigraphic studies on the corneal residence of a New Ophthalmic Delivery System (NODS): Rate of clearance of a soluble marker in relation to duration of pharmacological action of pilocarpine. *Br J Clin Pharmacol*. 1992;33(6):603-609.
64. Greaves JL, Wilson CG. Treatment of diseases of the eye with mucoadhesive systems. *Adv Drug Deliv Rev*. 1993;1: 349-383.
65. Schoenwald RD, Boltralik JJ. A bioavailability comparison in rabbits of two steroids formulated as high viscosity gels and reference aqueous preparations. *Invest Ophthalmol Vis Sci*. 1979;18: 61-66.
66. Unlu N, Ludwig A, vanoteghem M, Hincal AA. Formulation of carbopol-940 ophthalmic vehicles, and in vitro evaluation of the influence of simulated lacrimal fluid on their physicochemical properties. *Pharmazie*. 1991;46(11):784-788.
67. Wilson CG, Zhu YP, Frier M, Rao LS, Gilchrist P, Perkins AC. Ocular contact time of a carbomer gel, Geltears in man. *Br J Ophthalmol*. 1998;82(10): 1131 – 1134.
68. Wilson CG, McJury M, O'Mahony B, Frier M, Perkins AC. *Adv Drug Deliv Rev*. 1997;25: 91-102.
69. Budisantoso N, Frier M, Wilson CG. Lipophilic drug models for scintigraphic evaluation of drug delivery systems. *J Pharm Pharmacol*. 1997;49(suppl 4):49.
70. Kaempfer I. Chemistry of indium complexes. In: Deckart H, Cox PH, eds. *Principles of Radiopharmacology*. Gustav Fischer: Verlag Jena; 1987:128-135.

71. Wison CG, Zhu YP, Frier M. Scintigraphic study of ocular drug delivery. In: Perkins AC, Frier M, eds. *Nuclear Medicine in Pharmaceutical Research*. London; Taylor and Francis Ltd; 1999:115-132.
72. Greaves JL, Wilson CG, Rozier A, Grove J, Plazonnet B. Scintigraphic assessment of an ophthalmic gelling vehicle in man and rabbit. *Curr Eye Res*. 1990;9(5):415-420.
73. Zhu Y, Wilson CG, Meadows D, Olejnik O, et al. Dry powder dosing in liquid vehicles: ocular tolerance and scintigraphic evaluation of a perfluorocarbon suspension. *Int J Pharm*. 1999;191:79-85.
74. Hardberger RC, Hanna C, Boyd CM. Effects of drug vehicles on ocular contact time. *Arch Ophthalmol*. 1975;93:42-45.
75. Trueblood JH, Rossomondo RM, Carlton WH, Wilson LA. Corneal contact times of ophthalmic vehicles. *Arch Ophthalmol*. 1975;93:127-130.
76. Zaki I, Fitzgerald. P, Hardy JG, Wilson CG. A comparison of the effect of viscosity on the precorneal residence of solutions in rabbit and man. *J Pharm Pharmacol*. 1986;38: 463-466.
77. Fitzgerald P, Wilson CG, Greaves JL, Frier M, Hollingsbee D, Gilbert D, Richardson M. Scintigraphic assessment of the precorneal residence of a new ophthalmic delivery system (NODS) in man. *Int J Pharm*. 1992;83:177-185.
78. Snibson GR, Greaves JL, Soper NDW, Tiffany JM, Wilson CG, Bron AJ. Ocular surface residence times of artificial tear solutions. *Cornea*. 1992;11(4):288-293.
79. Kitazawa Y, Takahashi O, Matsumoto T, Iinuma T, Tateno Y. The effect of hydroxypropylmethylcellulose on corneal contact times of ophthalmic solution. *Acta Soc Ophthalmol Japan*. 1977;81:295-300.
80. Meseguer G, Gurny R, Buri P, Rozier A, Plazonnet B. Gamma scintigraphic study of precorneal drainage and assessment of miotic response in rabbits of various ophthalmic formulations containing pilocarpine. *Int J Pharm*. 1993;95:229-234.
81. Meseguer G, Gurny R, Buri P. Gamma scintigraphic evaluation of precorneal clearance in human volunteers and in rabbits. *Eur J Drug Metabol Pharmacokin*. 1993b; 18:190
82. Meseguer G, Buri P, Plazonnet B, Rozier A, Gurny R. Gamma scintigraphic comparison of eyedrops containing pilocarpine in healthy volunteers. *J Ocul Pharmacol Ther*. 1996;12(4):481-488.
83. Greaves JL, Wilson CG, Birmingham AT. Assessment of the precorneal residence of an ophthalmic ointment in healthy subjects. *Br J Clin Pharm*. 1993;35(2):188-192.
84. Gurny R, et al. Precorneal residence time in humans of sodium hyaluronate as measured by gamma scintigraphy. *Graefe's Arch Clin Exp Ophthalmol*. 1990;228: 510-512.
85. Stuart BO. Deposition of inhaled aerosols. *Arch Int Med*. 1973;131:60-73.
86. Mackey DWJ, Burch WM., Dance IG, Fisher, KJ, Willett, GD. The observation of fullerenes in a Technegas lung ventilation unit. *Nuc Med Commun*. 1994;15:430-434.
87. Jones JG, Lawler P, Crawley JCW, Minty BD, Hulands G, Veall N. Increased alveolar epithelial permeability in cigarette smokers. *Lancet*. 1980;1:66-68.

88. Hardy, J.G., Radiolabeling of pharmaceutical dosage forms. In: Sampson CB, ed. *Textbook of Radiopharmacy: Theory and Practice, 3rd ed.* Gordon and Breach Science Publishers: 1997.
89. Ito H, Pitcairn G, Hirst P, Newman S, Frier M, Perkins A. An in vivo study to assess the stability of technegas as a SPECT imaging agent for pulmonary drug formulations. *Respiratory Drug Delivery*. 2002;8:133-136. (was 89a)
90. Forge NI, Mountford PJ, O'Doherty MJ. Quantification of technetium-99m lung radioactivity from planar images. *Eur J Nuc Med*. 1993;20:10-15.
91. Fleming JS, Hashish AH, Conwa JH, Nassim MA, Holgate ST, Halson P, Moore E, Bailey AG, Martonen TB. Assessment of deposition of inhaled aerosol in the respiratory tract of man using three-dimensional multimodality imaging and mathematical modeling. *J Aerosol Med*. 1996;9:317-327. (was 90)
92. Smith D, Erskine D, Steele J, Hills D, Gazzard B. Comparison of nebulizer efficiency for aerosolizing pentamidine. *J Pharm Pharmacol*. 1992;44:7-9.
93. Hardy JG, Newman SP, Knoch M., Lung deposition from four nebulizers. *Respiratory Medicine*. 1993;87:461-465.
94. Thomas SHL, Doherty MJ, Page CJ, Nunan TO, Bateman NT. Which apparatus for inhaled pentamidine? A comparison of pulmonary deposition via eight nebulizers. *Eur Respir J*. 1991;4:616-622.
95. Chan H-K, Phipps PR, Gonda I, Cook P, Fulton R, Young I, Bautovich G. Regional deposition of nebulized hypodense nonisotonic solutions in the human respiratory tract. *Eur Respir J*. 1994;7:1483-1489.
96. McCallion ONM, Taylor KMG, Thomas M, Taylor AJ. The influence of surface tension on aerosols produced by medical nebulizers. *Int J Pharm*. 1996;129:123-136.
97. Everard ML, Hardy JG, Milner AD. Comparison of nebulized aerosol deposition in the lungs of healthy adults following oral and nasal inhalation. *Thorax*. 1993;48,1045-1046.
98. Chua HL, Collis GG, Newbury, AM, Chan K, Bower, GD, Sly PD, Le Souef PN. The influence of age on aerosol deposition in children with cystic fibrosis. *Eur Respir J*. 1994; 7:2185-2191.
99. Smith EC, Denyer J, Kendrick AH. Comparison of twenty-three nebulizer/compressor combinations for domiciliary use. *Eur Respir J*. 1995;8:1214-1221.
100. Smaldone GC. Deposition patterns of nebulized drugs: is the pattern important? *J Aerosol Med*. 1994;7(suppl 1):S25-32.
101. Newman SP, Pavia D, Garland N, Clarke, SW. Effects of various inhalation modes on the deposition of radioactive pressurized aerosols. *Eur J Resp Dis*. 1982;63(suppl 119):57-65.
102. Lawford P, McKenzie D. Pressurized aerosol inhaler technique: how important are inhalation from residual volume, inspiratory flow rate and the time interval between puffs? *Br J Dis Chest*. 1983;77:276-281.
103. Farr SJ, Rowe AM, Rubsamen R, Taylor G. Aerosol deposition in the human lung following administration from a microprocessor controlled pressurized metered-dose inhaler. *Thorax*. 1995;50:639-644.
104. Ashworth HL, Wilson CG, Sims EE, Wotton, PK, Hardy JG. Delivery of

propellant soluble drug from a metered-dose inhaler. *Thorax*. 1991;46:245-247.

105. Harnor KJ, Perkins AC, Wastie ML, Wilson CG, et al. Effect of vapour pressure on the deposition pattern from solution phase metered-dose inhalers. *Int J Pharmaceut*. 1993;95:111-116.

106. Leach C. Enhanced drug delivery through reformulating MDIs with HFA propellants—drug deposition and its effect on preclinical and clinical programs. In: Dalby RN, Byron PR, Farr SJ, eds. *Respiratory Drug Delivery V*. Buffalo Grove: Interpharm Press; 1996:133-144.

107. Melchor R, Biddiscombe, MF, Mak VHF, Short MD, Spiro SG. Lung deposition patterns of directly labeled salbutamol in normal subjects and in patients with reversible airflow obstruction. *Thorax*. 1993;48:506-511.

108. Biddiscombe MF, Melchor R, Mak, VHF, Marriott RJ, Taylor AJ, Short MD, Spiro SG. The lung deposition of salbutamol, directly labeled with technetium-99m, delivered by pressurized metered dose and dry powder inhalers. *Int J Pharmaceut*. 1993;91: 111-121.

109. Hardy JG, Everard ML, Coffiner M, Fossion J. Lung deposition of a nacystelyn metered-dose inhaler formulation, *J Aerosol Med*. 1993b;6:37-44.

110. Newman SP, Weisz AWB, Talaee N, Clarke SW. Improvement of drug delivery with a breath actuated pressurized aerosol for patients with poor inhaler technique, *Thorax*. 1991b;46:712-716.

111. Fergusson RJ, Lenney J, McHardy GJR, Crompton GK. The use of a new breath-actuated inhaler by patients with severe airflow obstruction. *Eur Respir J*. 1991;4:172-174.

112. Newman SP, Steed KP, Reader SJ, Hooper G, Zierenberg B. Efficient delivery to the lungs of flunisolide aerosol from a new portable hand-held multidose nebulizer. *J Pharma Sci*. 1996;85:960-964.

113. Barry PW, O'Callaghan C. The effect of delay, multiple actuations and spacer static charge on the in vitro delivery of budesonide from the Nebuhaler. *Br J Clin Pharmacol*. 1995;40:76-78.

114. Clark AR, Hollingworth AM. The relationship between powder inhaler resistance and peak inspiratory conditions in healthy volunteers - implications for in vitro testing. *J Aerosol Med*. 1993;6:99-110.

115. Pitcairn G, Lunghetti G, Ventura P, Newman S. A comparison of the lung deposition of salbutamol inhaled from a new dry powder inhaler, at two inhaled flow rates. *Int J Pharma*. 1994;102:11-18.

116. Newman SP, Hollingworth A, Clark, AR. Effect of different modes of inhalation on drug delivery from a dry powder inhaler, *Int J Pharma*. 1994a;102:127- 132.

117. Vidgren M, Paronen P, Vidgren P, Vainio P, Nuutinen J. In vivo evaluation of the new multiple dose powder inhaler and the Rotahaler® using the gamma scintigraphy. *Acta Pharmaceutica Nordica*. 1990.

118. Persson G, Olsson B, Soliman S. The impact of inspiratory effort on inspiratory flow through Turbuhaler in asthmatic patients. *Eur Respir J*. 1997;10:681-684.

119. Borgstrom L, Bondesson E, Moren F, Trofast E, Newman SP. Lung deposition of budesonide inhaled via Turbuhaler: A comparison with terbutaline sulphate in normal subjects. *Eur Respir J*. 1994:7:69-73.

120. Borgstrøm L, Newman SP, Weisz A, Morøn F. Pulmonary deposition of inhaled terbutaline: comparison of scanning gamma camera and urinary excretion methods. *J Pharm Sci.* 1992;81:753-755.
121. Thorsson L, Edsbeker S, Conradson T-B. Lung deposition of budesonide from Turbuhaler is twice that from a pressurized metered-dose inhaler P-MDI. *Eur Resp J.* 1994;7:1839-1844.
122. Lay JC, Berry, CR, Kim, CS, Bennett WD. Retention of insoluble particles after local intrabronchial deposition in dogs. *Journal of Applied Physiology*, 1995;79:921-929.
123. Agnew JE, Pavia D, Clarke SW. Factors affecting the 'alveolar deposition' of 5 μm inhaled particles in healthy subjects. *Clinical Physics and Physiological Measurement.* 1985;6:27-36.
124. Burch WM, Sullivan PJ, McLaren, CJ. Technegas-a new ventilation agent for lung scanning. *Nuc Med Commun.* 1986;7:865-871.
125. Bowden DH. Macrophages, dust, and pulmonary diseases. *Experimental Lung Research.* 1987;12: 89-107.
126. Proctor DF, Andersen I, Lundqvist G. Clearance of inhaled particles from the human nose. *Arch Intern Med.* 1973;131:132-139.
127. Bond SW, Hardy JG, Wilson CG. Deposition and clearance of nasal sprays. *Proceedings of the Second European Congress of Biopharmaceutics and Pharmacokinetics.* Salamanca. 1984:93-98.
128. Sakakura Y, Ukai K, Majima Y, Murai S, Harada T, Miyoshi Y. Nasal mucociliary clearance under various conditions. *Acta Otolaryngology.* 1983;96:167-173.
129. Lee SW, Hardy JG, Wilson CG, Smelt GJC. Nasal sprays and polyps. *Nuc Med Commun.* 1984;5:697-703.
130. Dondeti P, Zig H, Needham TE. Bioadhesive and formulation parameters affecting nasal absorption. *Int J Pharma.* 1996;127:115-133.
131. Aoki FY, Crawley JCW. Distribution and removal of human serum albumin-technetium 99m instilled intranasally. *Br J Clin Pharmacol.* 1976;3: 869-878.
132. Hardy JG, Lee SW, Wilson CG. Intranasal drug delivery by spray and drops. *J Pharm Pharmacol.*1985;37:294-297.
133. Newman SP, Morton F, Clarke SW. The nasal distribution of metered-dose inhalers. *J Laryngol Otol.* 1987;101:127-132.
134. Illum L, Jørgensen H, Bisgaard H, Krogsgaard O, Rossing, N. Bioadhesive microspheres as a potential nasal drug delivery system. *Int J Pharma.* 1987;39:189-199. (136 in text)
135. Newman SP, Steed KP, Hardy JG, Wilding IR, Hooper G, Sparrow RA. The distribution of an intranasal insulin formulation in healthy volunteers: effect of different administration techniques. *J Pharm Pharmacol.* 1994b;46:657-666.
136. Pennington AK, Ratcliffe JH, Wilson CG, Hardy JG. The influence of solution viscosity on nasal spray deposition and clearance. *Int J Pharma.* 1988;43:221-224.
137. Clark AR, Dasovich N, Gonda I, Chan. H-K. The balance and physical stability for inhalation protein powders: rhDNase as an example. In: Dalby RN, Byron PR, Farr SJ (eds.) *Respiratory Drug Delivery V.* Buffalo Grove: Interpharm Press; 1996:167-174.
138. Pouton CW, Uduehi AN, Lucas P. Pharmaceutical and biopharmaceutical

issues in gene delivery to the lung In: Dalby RN, Byron PR, Farr SJ (eds.) *Respiratory Drug Delivery V*. Buffalo Grove: Interpharm Press;1996:33-42.

139. Stoklin G and Pike VW. *Radiopharmaceuticals for Positron Emission Tomography*. Kluwer Academic Publishers., Dordrecht, 1993.
140. Aboagye EO, Luthra SK, Brady F, Poole K, Anderson H, Jones T, Boobis A, Burtles SS, Price P. Cancer Research UK procedures in manufacture and toxicology of radiotracers intended for pre-phase I positron emission tomography studies in cancer patients. *Br J Cancer*. 2002;86:1052-1056.
141. Del Guerra A, Belcari N. Advances in animal PET scanners. *Q J Nucl Med*. 2002;46:35-47.
142. Chatziioannou AF. Molecular imaging of small animals with dedicated PET tomographs. *Eur J Nucl Med Mol Imaging*. 2002;29(1): 98-114.
143. Lee Ds. Lee SK. Lee MC. Functional neuroimaging in epilepsy: FDG PET and ictal SPECT. *J Korean Med Sci*. 2001;16:689-696.
144. Raffel DM. Wieland DM. Assessment of cardiac sympathetic nerve integrity with positron emission tomography. *Nucl Med Biol*. 2001;28: 541-559.
145. Reske SN, Kotzerke J. FDG-PET for clinical use. Result of the 3rd German Interdisciplinary Consensus Conference. *Eur J Nucl Med*. 28:1707-1723 2001.
146. Willis MW. Ketter TA. Kimbrell TA. George MS. Herscovitch P. Danielson AL. Benson BE. Post RM. Age, Sex and laterality effects on cerebral glucose metabolism in healthy adults. *Psychiatry Res*. 114(1):23-37, 2002 Feb 15.
147. Okarvi SM. Recent progress in fluorine-18 labeled peptide radio-pharmaceuticals. *Eur J Nucl Med*. 2001;28:938.
148. Laverman P, Boerman OC, Corstens FM, Oyen WJG. Fluorinated amino acids for tumour imaging with positron emission tomography. *Eur J Nucl Med*. 2002;29:681-690.
149. Mankoff DA. Dunnwald LK. Gralow JR. Charlop A. Lawton TJ. Schubert EK. Tseng J. Livingstone RB. Blood flow and metabolism in locally advanced breast cancer: relationship to response to therapy. *J Nucl Med*. 2002;43:500-509.
150. Gennari A, Donati S, Salvadori B, Giorgetti A, Salvadori PA, Sorace O, et al. Role of 2-[18F]-fluorodeoxyglucose (FDG) positron emission tomography (PET) in the early assessment of response to chemotherapy in metastatic breast cancer patients. *Clin Breast Cancer*. 2000;1:156-163.
151. Dose J. Hemminger GE. Bohuslavizki KH. Therapy monitoring using FDG-PET in metastasic cervical cancer. *Lancet Oncol*. 2002;1:106.
152. Keiding S. Munk OL. Roelsgaard K. Bender D. Bass L. Positron emission tomography of hepatic first-pass metabolism of ammonia in pig. *Eur J Nucl Med*. 2001;28(12): 1770-17755.
153. Halldin C. Gulyas B. Farde L. PET studies with carbon-11 radioligands in neuropsycho-pharmacological drug develop-ment. *Curr Pharm Des*. 2001;7: 1907-29.
154. Tauscher J. Kapur S. Verhoeff NP. Hussey DF. Daskalakis ZJ. Tauscher-Wisniewski S. Wilson AA. Houle S. Kasper S. Zipursky RB. Brain serotonin 5-HT(1A) receptor binding in schizophrenia measured by positron emission tomography and [11C]WAY-100635. *Arch Gen Psychiatry*. 2002;59(6):514-520.

155. Doudet DJ. Jivan S. Ruth TJ. Holden JE. Density and affinity of the dopamine D2 receptors in aged symptomatic and asymptomatic MPTP-treated monkeys: PET studies with [11C]raclopride. *Synapse*. 2002;44:198.

156. Doze P. Elsinga PH. Maas B. Van Waarde A. Wegman T. Vaalburg W. Synthesis and evaluation of radiolabeled antagonists for imaging of beta-adrenoceptors in the brain with PET. *Neurochem Int*. 2002;40:145-155.

157. Brown LL, Kulkarni S., Pavlova QA, Koren AO, Mukhin AG, Newman AH, Horti AG. Synthesis and evaluation of a novel series of 2-chloro-5-((1-methyl-2-(s)-pyrrolidinyl)methoxy)-3-(2-(4-pyridinyl)vinyl)pyridine analogues as potential positron emission tomography imaging agents for nicotinic acetylcholine receptors. *J Med Chem*. 2002;45:2841-2849.

158. Schonbachler RD, Gucker PM., Arigoni M, Kneifel S, et al. PET imaging of dopamine transporters in the human brain using [(11)C]-beta-CPPIT, a cocaine derivative lacking the 2 beta-ester function. *Nucl Med Biol*. 29: 2002;19-27.

159. Rabiner EA. Wilkins MR. Turkheimer F. Gunn RN. De Haes JU. De Vries M. Grasby PM. 5-Hydroxytryptamine1A receptor occupancy by novel full antagonist 2-[4[4-(7-chloro-2,3-dihidro-1,4-benzdioxyn-5-yl)-1-piperazinyl]butyl]-1,2-benzisothiazol-3-(2H)-one-1,1-dioxide: a[11C][0-methyl-3H]-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY-100635) positron emission tomography study in humans. *J Pharmacol Exp Ther*. 2002;301:1144-1150.

160. Brady F, Luthra SK, Brown GD, Osman S, Aboagye E, Saleem A, Price PM. Radiolabeled tracers and anticancer drugs for assessment of therapeutic efficacy using PET. *Curr Pharm Des*. 2001;7(18): 1863-1892.

161. Ponomarev V, Doubrovin M, Lyddane C, Beresten T, et al. Imaging TCR-dependent NFAT-mediated T-cell activation with positron emission tomography in vivo. *Neoplasia*. 2001;3:480-488.

162. Price DT, Coleman RE, Liao RP, Robertson CN, Polascik TJ, DeGrado TR. Comparison of [18 F] fluorocholine and [18 F]fluoro-deoxyglucose for positron emission tomography of androgen dependent and androgen independent prostate cancer. *J Urol*. 2002;168(1):273-280.

163. Johnson SD, Welch MJ. Investigations into tumour accumulation and peroxisome proliferator activated receptor binding by F-18 and C-11 fatty acids. *Nucl Med Biol*. 2002;29: 211-216.

164. Ray P, Bauer E, Iyer M, et al. Monitoring gene therapy with reporter gene imaging. [Review] [91 refs]. *Semin Nuc Med*. 2001;31:312-320.

165. Wu JC, Inbushi M, Sundaresan G, Schelbert HR, Gambhir SS. Positron emission tomography imaging of cardiac reporter gene expression in living rats. *Circulation*. 2002;106:180-183.

166. Iozzo P, Osman S, Glaser M, et al. In vivo imaging of insulin receptors by PET: preclinical evaluation of iodine-125 and iodine-124 labeled with human insulin. *Nucl Med Biol*. 2002;29:73-82.

167. Price JC, Kelley DE, Ryan CM, et al. Reynolds CF 3rd. Evidence of increased serotonin-1A receptor binding in type 2 diabetes: a positron emission tomography study. *Brain Res*. 2002;927(1):97-103.

PLEASE NOTE:

This is a double lesson: Volume 10, Lessons 3 and 4. Together these lessons are worth a total of 0.6 CEUs (6.0 credit hours).

- You must complete and return two answer sheets in order to obtain full credit.
- Submit one answer sheet per lesson.
- There are 30 questions per lesson.
- Complete one answer sheet for Lesson 3. Questions 1-30.
- Complete one answer sheet for Lesson 4. Questions 31-60.

QUESTIONS

1. The most widely used means of controlling risk during the conduct of clinical trials are:
   a. Economic
   b. Regulatory
   c. Advisory
   d. Technical

2. The Institutional Review Board/ Independent Ethics Committee should
   a. Have a maximum membership of 5
   b. Have a membership totally independent of the trial site
   c. Be under the chairmanship of a lay member
   d. Have an exclusively clinical and scientific membership

3. The ICH/GCP Guidelines are
   a. Mandatory in all countries
   b. Are recommended for adoption in the United States
   c. Have not been accepted in the European Union
   d. Allow the waiver of Good Manufacturing Practices during the manufacture of Clinical trial materials

4. A trial protocol
   a. Must include all information relevant to the trial without reference to other documents
   b. Must identify the trial sponsor and study physician
   c. Need not contain background information on non-clinical studies
   d. Cannot be amended

5. An effective radiation dose of between 0.2 and 2 mSv
   a. Causes a cancer risk of up to 1 in 10,000
   b. Causes a cancer risk of up to 1 in 100,000
   c. Causes a cancer risk greater than the natural annual risk
   d. Is unacceptable for a clinical trial

6. Dynamic planar gamma camera imaging involves
   a. Rotation of the gamma camera 360° around the patient
   b. Filtered back projection of the data
   c. Recording data in a large matrix size
   d. A consecutive series of image frames

7. Image data may be quantified by
   a. Defining regions of interest for the extraction of count rates
   b. Adding background counts to image data
   c. Counting the number of pixels in the image
   d. Extracting the times of the individual image frames

8. SPECT imaging is performed by
   a. Filtered back projection of planar image data obtained by a rotating gamma camera
   b. Obtaining the geometric mean of opposing planar views
   c. Defining ROIs from dynamic images
   d. Dynamic gated acquisition

9. Quality assurance of image data
   a. Is only necessary for clinical diagnostic studies

b. Can be carried out using standard image data sets
c. Is not possible for image software
d. Is only necessary for radionuclide calibrators

10. Scintigraphic visualization of drug delivery
    a. Will only demonstrate site of drug release
    b. Cannot be quantified from planar images
    c. Can be used to quantify in vivo release, biodistribution and kinetics
    d. Cannot be performed by clinical gamma camera systems

11. Prior to using a Medicinal Product for investigational purposes, a sponsor will be required to:
    a. File an acceptable IND submission as required by the US FDA for distribution
    b. File an acceptable IND submission as required by the US FDA for importation
    c. Obtain authorisation for manufacture, assembly or importation in the EEC
    d. All of the above

12. Good Manufacturing Practice
    e. Is part of Quality Assurance
    f. Only applies to routine manufacture
    g. Only applies to investigational medicinal products
    h. Replaces the need for a quality management system

13. Protocols for manufacture of investigational medicinal products
    a. Should include mandatory, invariable processing instructions
    b. Can allow variations in procedure if variations are approved and recorded
    c. Need only specify starting materials
    d. Need only specify finished products and release criteria

14. During the manufacture of investigational medicinal products, it is acceptable for
    a. One operator to perform the whole procedure
    b. The same individuals to be involved with production and quality control
    c. Product release criteria to be varied
    d. Non-validated procedures to be adopted

15. Radiopharmaceuticals for GI transit studies should
    a. Be validated for use before administration
    b. Not be absorbed through the GI mucosa
    c. Have a physical half-life suited to the time of transit being studied
    d. All of the above

16. Esophageal transit studies:
    a. Can show the position of retention of a solid dosage form in the esophagus
    b. Should only be employed if x-ray studies are not available
    c. Are only possible for capsule formulations
    d. Should only be performed with the subject in the erect position

17. Neutron activation of oral dosage forms:
    a. Requires the formulation to be irradiated in a neutron source, such as a nuclear reactor
    b. Can be undertaken on any solid dose form without the need to add any foreign material
    c. Is not suitable for the study of colonic drug delivery
    d. Will only produce gamma emitting radionuclides

18. Gastric emptying studies of oral formulations
    a. Cannot show liquid and solid emptying simultaneously
    b. Can demonstrate both gastric emptying and reflux of a formulation
    c. Cannot be undertaken if esophageal transit or small intestinal transit are being measured
    d. Cannot demonstrate coating of a liquid or dispersed formulations

19. The study of targeted colonic drug delivery
    a. Is best studied with ^{99m}Tc radiopharmaceuticals
    b. Will not show colonic transit of formulations once they have dispersed in the colon
    c. Will not allow quantification of regional drug delivery
    d. Can be carried out over 72 hours using ^{111}In radiopharmaceuticals

20. Drug delivery to the eye is frequently unsuccessful because of
    a. Slow precorneal clearance
    b. High corneal absorption
    c. Drug losses via the conjunctival route
    d. Non-selective absorptive properties of the cornea

21. After topical application of drugs to the cornea, the amount reaching the aqueous humor is typically
    a. >10%
    b. >5%
    c. <5%
    d. >20%

22. The most radiosensitive part of the eye is
    a. The cornea
    b. The retina
    c. The lens
    d. The conjunctiva

23. A suitable lipophilic radiotracer for an emulsified ophthalmic formulation is
    a. ^{99m}Tc DTPA
    b. ^{99m}Tc pertechnetate
    c. ^{99m}Tc isonitrile
    d. ^{99m}Tc mucin

24. The proportion of patients unable to use eyedropper bottles correctly is
    a. <20%
    b. >20%
    c. >50%
    d. >40%

25. The optimal size for particle deposition in the lung is
    a. 5μm
    b. Between 0.5 and 5 μm
    c. >10μm
    d. < 0.2 μm

26. Half-time of clearance of ^{99m}Tc-DTPA from the healthy lung is
    a. < 10 minutes
    b. About 30 minutes
    c. About 1 hour
    d. More than 4 hours

27. Lung deposition of drug from metered-dose inhalers containing drug suspension is
    a. About 20% in normal subjects
    b. Higher in asthmatics than in non-asthmatics
    c. Concentrated in the peripheral airways in asthmatic subjects
    d. Unaffected by user technique

28. Scintigraphic imaging of the deposition of drugs in the lung is only applicable to the use of:
    a. Nebulizers
    b. Metered-dose inhalers
    c. Solid-powder inhalers
    d. All of the above

29. Scintigraphic drug delivery studies can give information on
    a. Site of drug release from the formulation
    b. Site of drug absorption
    c. Metabolic fate of drugs
    d. Excretion of unchanged drugs and their metabolites

30. Scintigraphic studies of drug metabolism are difficult because of
    a. Limited availability of gamma-emitting radionuclides for incorporation into drug molecules
    b. Very short half lives of positron-emitting radionuclides, limiting available synthesis time for labeled drug molecules
    c. Unwanted effects on metabolism of drugs through the presence of "foreign" atoms as radiolabels.
    d. All of the above

****

BEGIN TEST FOR VOLUME 10, LESSON 4

31. During development of drugs and drug delivery systems, specific advantages offered by PET include
    a. The ability to determine long term metabolic fate
    b. The ability to demonstrate sites of drug absorption
    c. The ability to incorporate the tracer at an early stage in production of the formulation
    d. The ability to conduct acute toxicity studies

32. Planar gamma camera images may be used for:
    a. Imaging the site of release and GI distribution of orally administered formulations.
    b. Quantifying regional distribution of a radiolabeled drug or molecule.
    c. Demonstrating tumor uptake of targeted agents.
    d. All of the above.

33. What is a major advantage of PET over SPECT for imaging drug distribution?
    a. The short physical half-life of positron emitters.
    b. The low cost of equipment for PET imaging
    c. The well-established chemistry for radiolabeling with ^{99m}Tc.
    d. The use of positron emitting elements such as O, N, C and Fl.

34. A Radioactive Drug Research Committee operates under the authority of which of the following agencies?
    a. FDA

b. EPA
c. DOT
d. NRC

35. The function of a Radioactive Drug Research Committee is to oversee research projects (involving administration of a radioactively labeled drug) that are intended to obtain information on:
a. Metabolism of a radioactive drug
b. Human physiology, pathophysiology, or biochemistry
c. Safety and efficacy of a radioactive drug
d. A and B only.

36. Which of the following statements is inconsistent with the principles of Good Clinical Practices?
a. Informed consent should be obtained from each trial subject.
b. The interests of, and benefits to, science and society should prevail over the rights, safety, and well being of the trial's subjects.
c. Privacy and confidentiality of trial subjects' records should be respected and protected.
d. Investigational products should be manufactured, labeled, and stored according to current Good Manufacturing Practices.

37. The main purpose of the Institutional Review Board is to assure that _____.
a. The trial protocol is based on valid statistical principles
b. Investigators meet the trial's training and experience requirements
c. The rights, safety, and well being of the trial's subjects are safeguarded
d. The trial's budget is adequate to meet its stated purpose

38. Which of the following is the radiation dose limit for the lens of the eye, as set by the FDA for a single dose administration of a radioactive drug?
a. 30 mSv
b. 50 mSv
c. 75 mSv

39. U.S. regulations for current Good Manufacturing Practices are found in which of the following locations?
a. 10 CFR Parts 67-68
b. 21 CFR Parts 210-211
c. 49 CFR Parts 304-305
d. 70 CFR Parts 17-18

40. Which of the following best completes this sentence? Radioactivity versus time curves may be generated _____.
a. from a series of dynamic images
b. if the patient is consistently imaged in the same anatomical position
c. to assess the rates of uptake, dissolution, and spread of a radioactive dosage form
d. All of the above statements are true.

41. Which of the following compounds is not commonly used to adsorb small volumes of a radiopharmaceutical solution when preparing a radioactive capsule dosage form?
a. Sodium chloride
b. Lactose
c. Sucrose
d. Calcium phosphate

42. Which of the following is not a factor that contributes to esophageal adhesion of an orally administered pharmaceutical dosage form?
a. Size of the dosage form
b. Shape of the dosage form

c. Adhesive properties of the dosage form's surface coating
d. Color of the dosage form's surface coating

43. For study of ocular drug suspensions, which of the following agents is recommended for labeling with Tc-99m DTPA?
a. Antimony colloid
b. Sulfur colloid
c. Micronized carbon
d. Aggregated albumin

44. Using information obtained from Table 2 of this CE lesson, which of the following statements is true?
e. Normal saline is retained in contact with the cornea to the same extent as 0.5% hydroxyethylcellulose (HEC).
f. Higher concentrations of HEC are retained in contact with the cornea longer than lower concentrations.
g. Lower concentrations of HEC are retained in contact with the cornea longer than higher concentrations.
h. None of the above are true.

45. Following administration, nebulized solutions containing Tc-99m DTPA are typically deposited either in the lungs or swallowed. The swallowed portioned of the dose is eliminated via which of the following routes?
a. urine
b. sweat
c. feces
d. exhaled vapors

46. Which of the following factors pertinent to nasal drug delivery can be investigated using gamma scintigraphy?
a. site of drug deposition
b. extent of drug deposition
c. residence time of the drug in the nasal cavity
d. all of the above

47. Which of the following methods can enhance drug delivery via the nasal route?
a. by altering the drug formulation to prolong the drug in the nasal cavity only
b. by incorporating penetration enhancers into the drug formulation only
c. both (a) and (b)
d. neither (a) or (b)

48. In the generator system where Ge-68 is the parent nuclide, which of the following nuclides is the daughter of Ge-68 decay?
a. Ga-68
b. Se-68
c. Ar-68
d. Mn-68

49. Which of the following is true about scintigraphy?
I. It involves imaging the biosdistribution of a radiopharmaceutical
II. It does not permit correlation between observed pharmacology effects and the precise site of delivery
III. It provides data on the nature and characteristics of products
IV. It facilitates drug-targeting studies.
a. I and II only
b. II and III only
c. I, III, and IV only
d. I, II, III, and IV

50. Scintigraphic study is no longer accepted by regulatory authorizes as supporting evidence in product registration dossiers like Investigational New Drug Applications (INDs) ilr Bew Dryg Applications (NDAs).

a. True
b. False

51. Control of risk in human studies may be achieved in a variety of ways. Which of the following is the most widely used for clinical trials?
a. Economic
b. Regulatory
c. Technical solutions
d. Advisory solutions

52. Regulations govering the Radioactive Drug Research Committee (RDRC) are found in
a. 21 CFR Part 312
b. 21 CFR Part 361
c. 21 CFR Part 50
d. 21 CFR Part 56

53. Basic radiopharmaceutical research is used to obtain information regarding the _____ of the radioactive drug.
I. Metabolism
II. Kinetics
III. Distribution
IV. Localization
a. I and II only
b. II and III only
c. I, III, and IV only
d. I, II, III, and IV

54. What is the single dose limit for radiation dose set by the FDA in the RDRC regulations for active blood-forming organs?
a. 3 rem
b. 30 rem
c. 3 mSV
d. none of the above.

55. What is the annual and total dose commitment dose limit for radiation dose set by the FDA in the RDRC regulations for the lens of the eye?
a. 5 rem
b. 50 rem
c. 5 mSV
d. none of the above.

56. Which of the following is not a parameter for a dynamic acquisition study?
a. Matrix size
b. Frame time
c. Total number of frames
d. Predefined time

57. Which of the following arc examples of a dual radionuclide study?
I. Simultaneous monitoring of a drug and a delivery device
II. An oral dose form and a dietary component
III. Liquid and solid phases of an oral formulation
IV. A targeting moiety and a cytotoxic moiety.
a. I and III only
b. II and IV only
c. II, II, and IV only
d. I, II, III, and IV

58. Which of the following needs to be considered when quantifying data from scintigraphic images?
I. Time and duration of image acquisition
II. Background substraction
III. Decay correction
IV. Gamma ray attenuation
V. Activity time curves
a. I and III only
b. II and IV only
c. II, II, and V only
d. I, II, III, IV, and IV

59. What are some of the main variables affecting the collection of image data using rotating gamma cameras?

I. Number of angular increments for data collection
II. Color of image matrix
III. Choice of collimator
IV. Detector uniformity correction
V. Activity time curves

a. I and III only
b. II and IV only
c. I, III, and IV only
d. I, II, III, and IV

60. Of the following, which is not true of SPECT imaging? Images may be displayed as _____.

a. Octagonal slices
b. Axial slices
c. Coronal and sagittal slices
d. Oblique cuts through any chosen plane