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IMAGING AND ITS APPLICATIONS TO DRUG DELIVERY

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ABSTRACT

Non-invasive, gamma, and positron scintigraphy can be used to obtain information on drug absorption, distribution, and elimination, as well as, on drug delivery and release from special formulations and controlled delivery devices. To accomplish this, appropriate gamma and/positron emitting radiotracers must be developed which can be incorporated into the formulation or the drug delivery device. Since these studies are non-invasive, they can be conducted in valuable experimental animals, e.g. primates as well as in humans. There are numerous approaches to aid in defining delivery, release, and absorption of compounds. Beginning with conventional radiotracers, progressing to neutron activated compounds, and finally to compounds radiolabeled with halogens or positron emitters one can chose the approach which will provide the information needed in the most reasonable way. Non-invasive imaging allows one to do this in a more physiological state in both pre-clinical and clinical applications.

INTRODUCTION

The use of gamma scintigraphy has expanded in recent years to include applications in drug delivery. This area of non-invasive imaging and delivery has been the subject of numerous papers, reviews, studies, and courses[1,2,3]. This review will cover a number of topics including techniques and instrumentation. Advantages and limitations of scintigraphy will be discussed and examples of applications presented. This presentation will only focus on non-invasive radioisotope applications, however, other related modalities such as autoradiography are applicable to research in this area.

The use of scintigraphy for drug delivery studies is based upon the incorporation of a radiotracer within a formulation, drug delivery device, or drug. Information on the physical relationship between this label and the formulation, device, or drug must be provided. This is often a simple task since the radiotracer is encapsulated in a delivery device which is sealed, or a complex task if the radioisotope is coupled to a compound. For example, the tagging of a compound could change its pharmacokinetic properties making the data more difficult to interpret. Scintigraphy has a number of strengths based upon its non-invasive nature. Preclinical studies in conscious animals can be conducted under more normal physiological conditions than studies with anesthetized subjects. Also higher order animals such as non-human primates can be used more readily. Results of studies in non-human primates are often more reliable predictors of expected human results. Furthermore, chronic studies can be conducted permitting the use of each animal as its own control, thereby, reducing biological variability. A limiting factor in human studies is

the radiation dose to the patient and this limits many dynamic delivery applications to preclinical studies. Most experimental protocols, however, can be adapted for clinical studies in man and at times going directly to human studies is the preferred route.

Protocols employing scintigraphy must be well planned since the techniques are complex and costly involving expensive instrumentation and requiring highly trained personnel. Experiments often involve a team of scientists including radiochemists, imaging specialists, and experts in pharmaceutics. Personnel capable of interpreting inherent scintigraphic problems and devising protocols to deal with sources of error such as motion, scatter, and attenuation are essential.

The following sections will focus on instrumentation, collection of data, radiolabeling of dosage forms and compounds, established models of drug delivery in the gastrointestinal tract, and examples of unique applications to pharmaceutical development. An excellent course in gamma scintigraphy was conducted at the AAPS Eastern Regional Meeting and it is hoped that it is offered again for those desiring additional basic information on this topic[3].

INSTRUMENTATION AND BASIC PHYSICS

Scintigraphy involves the detection of gamma radiation emitted from a radioisotope. The radioisotope is normally incorporated into a dosage form or compound and administered to the subject via various routes of administration. The gamma radiation emanating from the subject, is collimated and then detected by a crystal. The energy is transformed to scintillations of light and amplified so that the information can be digitized in a quantifiable manner.

Scintigraphy involves two types of radiation, single-photon gamma emitters and positrons. Single photon emitters such as ^{123}I emit radiation in a 360 degree radius and only a portion of that radiation is collimated, viewed, and quantitated. Positron radiation results in the emission of a positron which collides with an electron and annihilates producing two photons emitted back to back at 180 degrees. This phenomena allows for coincidence counting which can be used to provide precise quantification. Also single photon emission computed tomography (SPECT) and positron emission tomography (PET) can be used to correct for attenuation problems which occur in planar imaging. Positron tomography involves expensive instrumentation and the availability of a cyclotron to produce positron emitters. Approximately 30 centers in the United States have this capability and experiments cost approximately $2000 per isotope produced. As will be illustrated in the applications section the use of isotopically labeled ^{11}C positron emitters can provide definitive information on delivery of compounds.

The most common use of scintigraphy involves planar imaging using both large and small view cameras. Tomographic cameras have had limited applications for delivery studies but operate on the same basic principles as planar scintigraphy. The use of SPECT and PET may resolve some technical issues but more precise quantitation of the data is often not needed. Differences from planar imaging include ring systems or rotation of the planar head to provide for tomographic capabilities. In addition crystals, collimators, and computers must be tailored to the type of emission studied. A more thorough review of SPECT and PET instrumentation is provided by Freeman. et al[4].

The planar camera consists of NaI(Tl) crystals 15" diameter, 37 or 75 photomultiplier tubes for amplification, suitable for energies of 60-360 Kev with spatial resolution of around 0.5 cm. The large field cameras can normally encompass the majority of the thoracic and gastrointestinal cavities of a dog or non-human primate. The gamma rays are first collimated to define the field of view, the activity translated into its x and y axis and image reconstructed. While image quality and good spatial resolution is desired for defining regions of interest (ROI) the strength of nuclear imaging comes from the ability to measure the activity in those defined regions. Sophisticated nuclear imaging computers can then be used to define areas, calculate time activity curves and quantitate the activity within ROIs. Depending on the activity administered to the subject dynamic images can be obtained within very short time frames (seconds to minutes) so that precise delivery information can be obtained.

RADIONUCLIDES AND LABELING

The most common radionuclides used in drug delivery imaging are listed in Table 1.

Various technetium complexes have been used for pharmaceutical delivery studies including ^{99m}Tc-HDP for p.o., ^{99m}Tc-DTPA for aerosols, and ^{99m}Tc-sulfur colloid for suspensions. ^{111}In has been used in ophthalmic suspensions, capsules, tablets, and liposomes. Applications involving compounds are generally limited to the ability to synthesize stable iodinated compounds usually by reacting with phenolic and/or amino residues. It should be emphasized that labeling with all of the radionuclides mentioned other than ^{11}C usually results in a complex or analog of the parent compound which may

TABLE 1

RADIONUCLIDES USED IN PHARMACEUTICAL PREPARATIONS

Nuclide	Half-life	Energy (KeV)
Technetium-99m	6.0 hrs	140
Indium-111	2.8 days	173
		247
Iodine-123	13 hrs	159
Iodine-131	8 days	364
Carbon-11(cyclotron)	20 mins	511

affect its distribution and metabolism. As will be illustrated later, with careful consideration of the analogs made, ^{123}I or ^{131}I labeled compounds can be used in specific cases to provide useful information on the suitability of drug delivery formulations or devices.

Techniques also exist for neutron activation utilizing the isotopes ^{139}Ba, ^{171}Er, and ^{153}Sm whose half-lives are 83 minutes, 7.5 hours, and 47 hours respectively. Their energies are all within the ranges of detection described. To produce the appropriately activated daughter the stable parent isotope must be incorporated into the formulation prior to manufacturing and then exposed to a neutron source at a reactor facility. The radioactive formulation can then be administered to the subject. The stable isotope which is incorporated into the formulation must have a high natural abundance, a high neutron capture cross section, be chemically inert, and not cause toxic effects. Its main

advantages are its application to the labeling of complex dosage forms and use in larger scale formulations. The advantages of this technique is just being realized and will most likely expand.

MODELS OF DELIVERY IN THE GASTROINTESTINAL TRACT

There are numerous articles on drug delivery in the gastrointestinal tract[4,5,6,7,8,9]. The importance of evaluating the *in vivo* behavior of dosage forms comes to light due to the varied absorption of compounds in specific areas of the gastrointestinal tract[10], and because of the upsurge in controlled and slow release devices. Additionally, there is extensive work on developing new vehicles to transport lipophilic compounds. Scintigraphy has been combined with radiotelemetry[11,12] and plasma profiles[13] to allow for comparisons of physiological conditions, *in vivo* and *in vitro* kinetics of release, and absorption. The release of compounds and absorption in regions of the gastrointestinal tract depends upon physiological conditions such as agitation and pH as well as the properties of the compound[14]. Establishment of correlations between *in vitro* dissolution and *in vivo* release is important and will be further expanded upon in the next section. There are numerous factors which affect the motility of the gastrointestinal tract, the most important being the intake of food[15]. Studies assessing the fed and fasted state have added considerable information on the behavior of delivery forms. Distinct differences can be seen (Figures 1-4) depending on the state of food intake and the delivery form[7].

Correlation studies between *in vitro* and *in vivo* release profiles using scintigraphy and deconvolution analysis of blood levels show good agreement. The following studies (Figure 5) conducted by Davis et.al with single unit

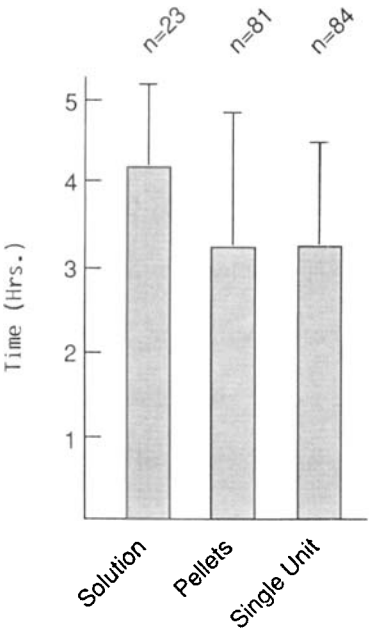

FIGURE 1

Small Intestinal Transit of Solutions Pellets and Single Units Under Different Feeding Conditions

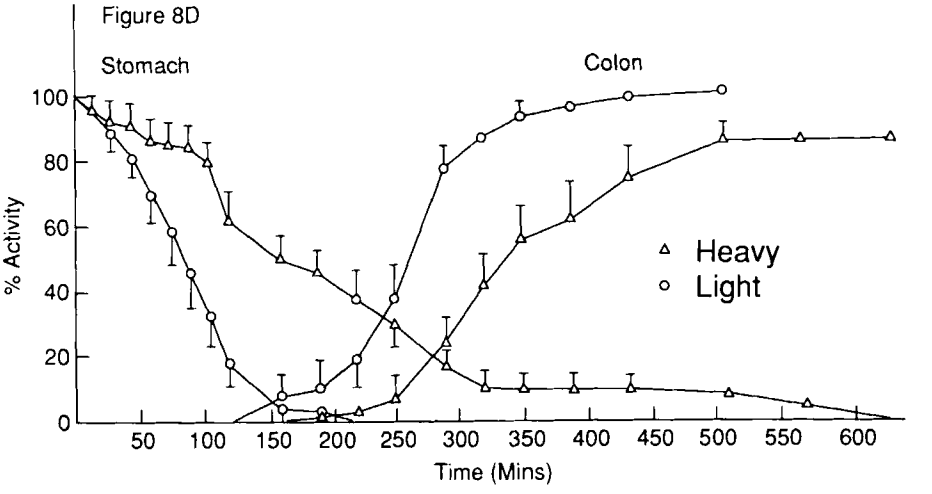

FIGURE 2

Gastrointestinal Transit of Tablets (3mm, 4mm, 5mm Tablets) - Pooled Data Showing Effect of Meal Size (n=9)

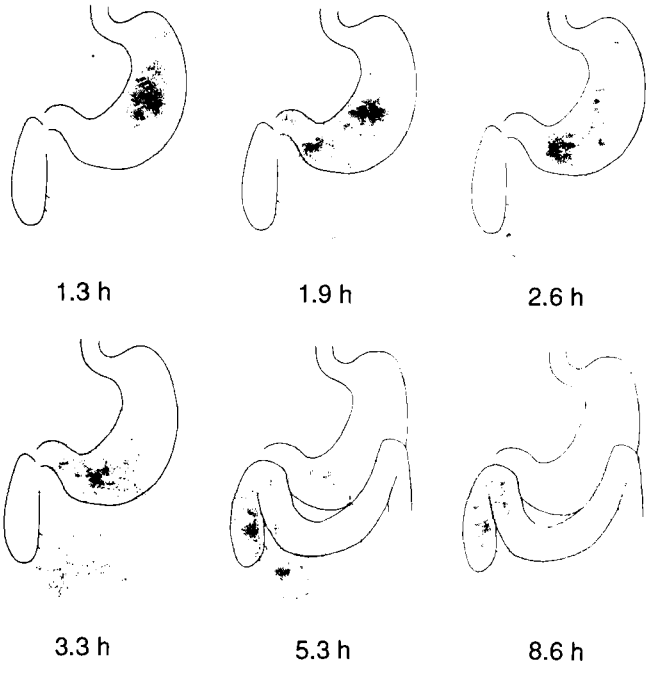

FIGURE 3

Gastrointestinal Transit of Pellets Following a Heavy Breakfast

matrix systems where release should be largely independent of pH and agitation conditions show good correlation between *in vitro* and *in vivo* release[14].

An excellent list of questions pertinent to gastrointestinal transit of drug delivery systems was compiled by Davis and Hardy[5]. An example of an approach to correlate *in vitro* data with release in the gastrointestinal tract of a labeled compound from a delivery device will be covered in the following section.

APPLICATIONS TO PHARMACEUTICAL DEVELOPMENT

Three examples of imaging applications to drug development will be reviewed: first, the use of scintigraphy to follow the release of a lipophilic

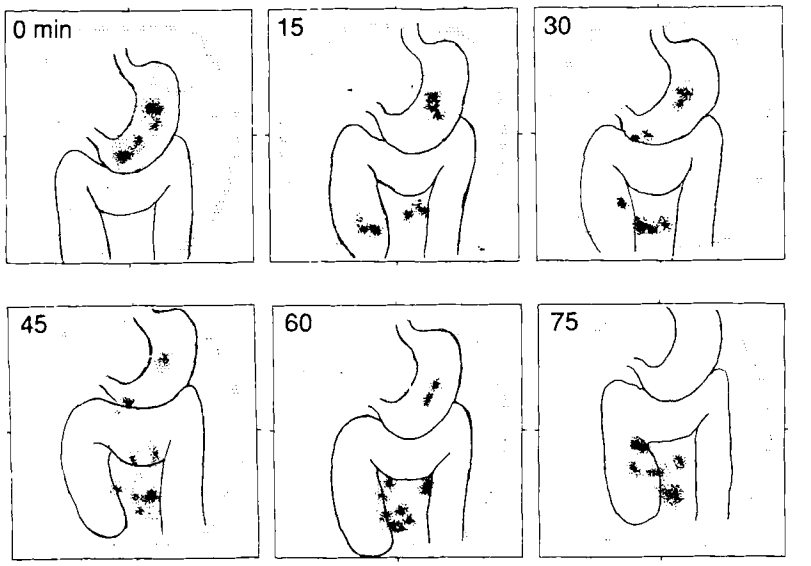

FIGURE 4A

Gastrointestinal Transit of 10 Small Tablets Size 4mm, Following a Light Breakfast

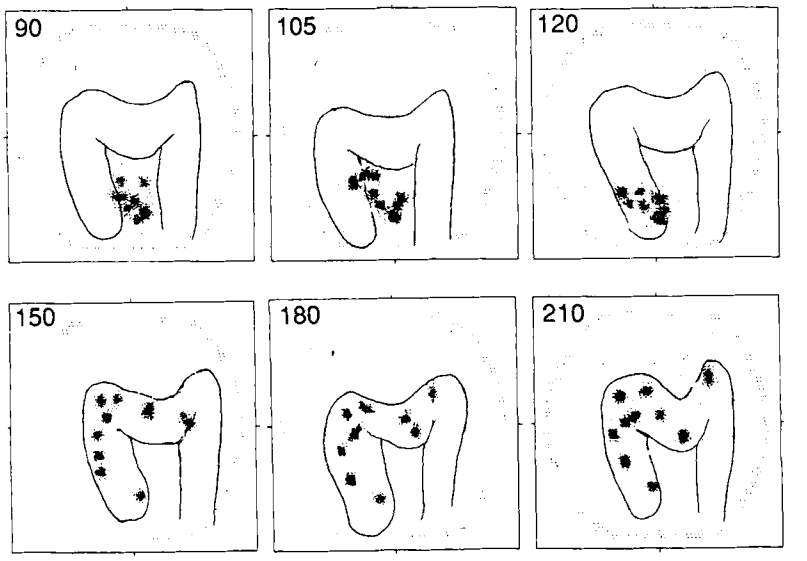

FIGURE 4B

Gastrointestinal Transit of 10 Small Tablets Size 4mm, Following a Light Breakfast

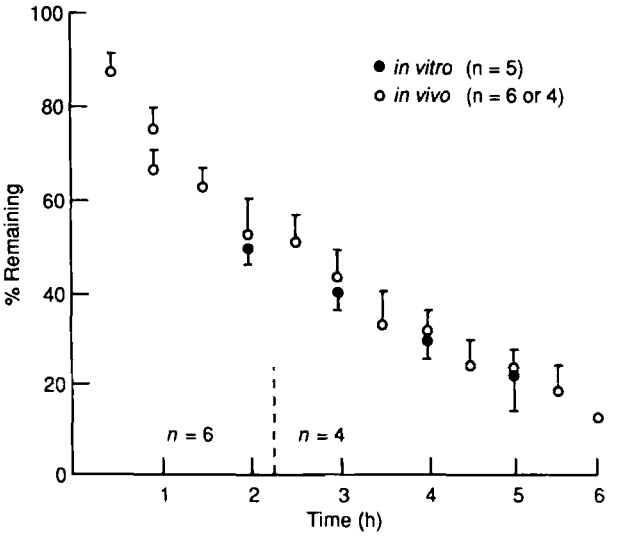

FIGURE 5

Correlation of *In Vitro* And *In Vivo* Release With A Single Unit Matrix System

compound from a controlled release device; second, the use of positron emission tomography to establish whether a compound of interest is delivered to its intended target; and lastly, the use of both an iodinated and ^{11}C ligand compound for examining drug delivery.

The objective of the first study[16] was to follow the release of a compound from a delivery device and correlate this release with the position in the gastrointestinal tract. The compounds to be used in this controlled delivery device are lipophilic and the route of administration is oral. Previous work in our laboratory had resulted in the successful iodination of a number of lipophilic radioligands with characteristics very similar to the compounds of interest. To verify whether one of these compounds would be useful as a marker of *in vivo* release, *in vitro* dissolution experiments were conducted (Figure 6).

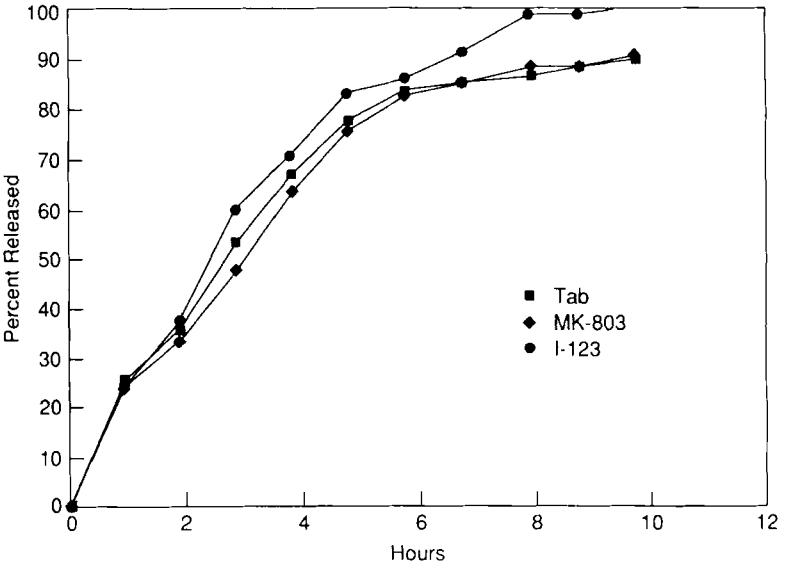

FIGURE 6

Drug/I-123 Release From Drug Delivery Device

As can be seen from this data the pharmaceutical labeled with ^{123}I exhibited the same release rates *in vitro* as the intended compound to be incorporated into this device. Since this marker showed similar *in vitro* dissolution characteristics to the drug of interest, *in vivo* studies were conducted in conscious beagle dogs in fasted and fed states. Initial studies using a radiolabeled meal were conducted to outline the anatomy of each animal prior to the studies with the radioligand/device.

The drug release study was done using geometric mean analysis[17] to assist in quantification of release. This technique is one way to correct for attenuation of the radiation through the animal. As shown the release rates in the fed and fasted states are remarkably different (Figures 7 and 8).

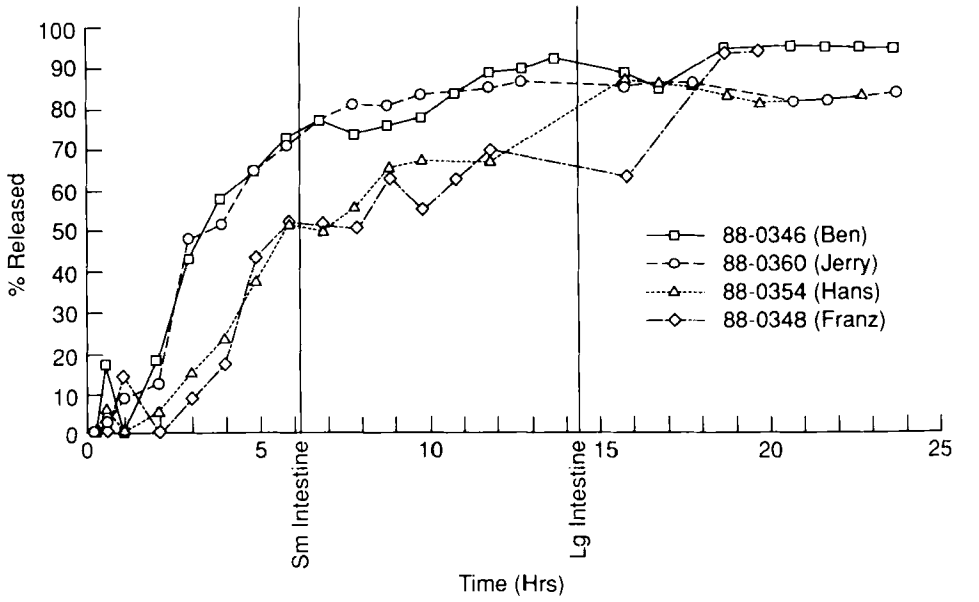

FIGURE 7

Drug Release In Fed Dogs

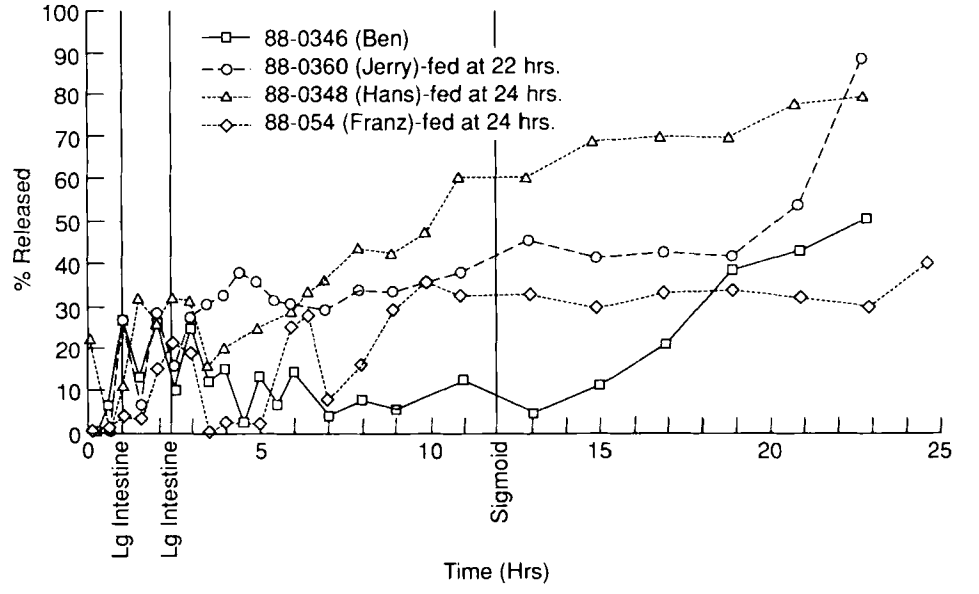

FIGURE 8

Drug Release In Fasted Dogs

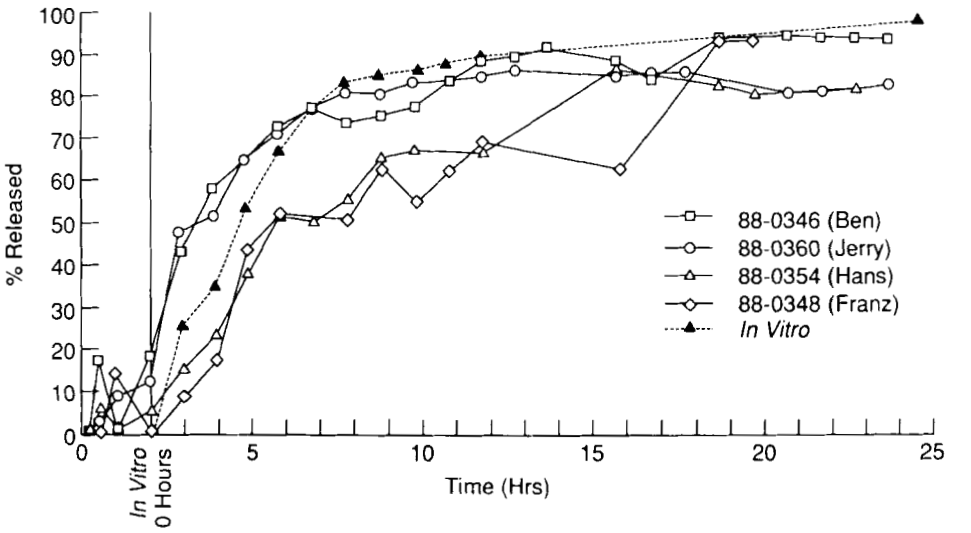

FIGURE 9

Comparison of Fed Dogs and *In Vitro* Studies

Additionally, the release rate in the fed state is very similar to the *in vitro* dissolution rate with an induction phase of 1-2 hours (Figure 9). This allows for further *in vitro* assessment of compounds in that system. Once a final configuration of the controlled delivery device is established, scintigraphy can then be used in a clinical trial to verify the release rate.

A second example of the utility of imaging is illustrated by the following experiment. A question was raised whether the compound MK-801 could penetrate the targeted area of clinical interest, the CNS, since one of the indications of this class of compounds is for treatment of stroke. Since blood flow in the region of a stroke is severely compromised the extent of penetration of the compound into this region was investigated. As can be seen in Figure 10, penetration of the ^{11}C compound does occur after IV administration. In fact the compound appears to diffuse throughout the penumbra of the stroke.

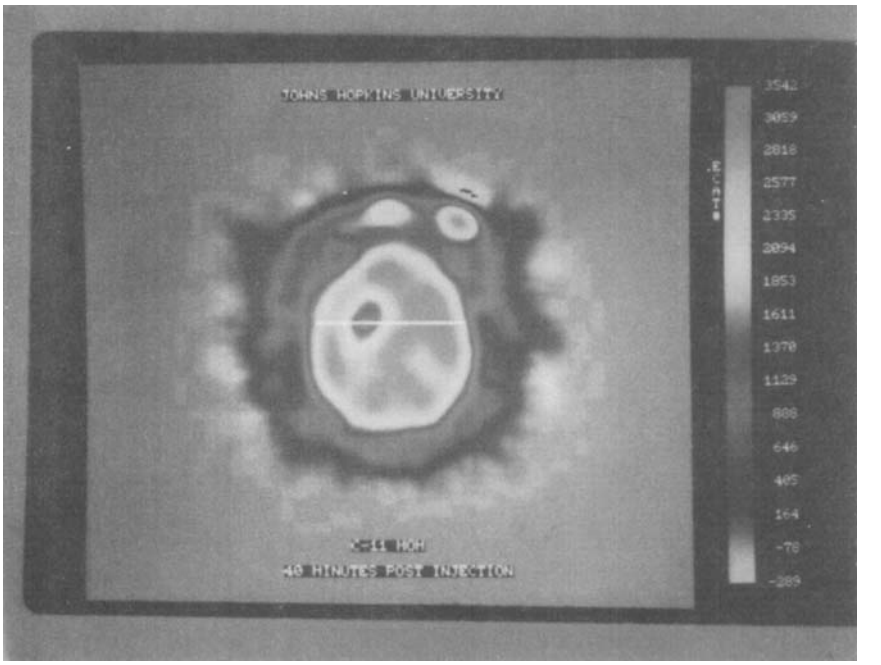

FIGURE 10

C-11 Compound Delivery to the Ischemic Area of the Brain

The last example consists of a set of experiments designed to examine the distribution of a drug/emulsion complex[18,19]. MK-329, a potent CCK-A antagonist, has been proposed as a potential treatment for various gastrointestinal disorders. A number of these disorders require parenteral administration. This compound, being highly lipophilic could not be incorporated into any standard formulations approved for human use, therefore, an emulsion system was formulated by Pharmaceutical Research and Development. Since large particle size emulsions are taken up rapidly by the liver, a microfine emulsion with a mean diameter of 212nm was tested to determine if the small particle size would result in reduced uptake by the liver. Metabolism to an inactive

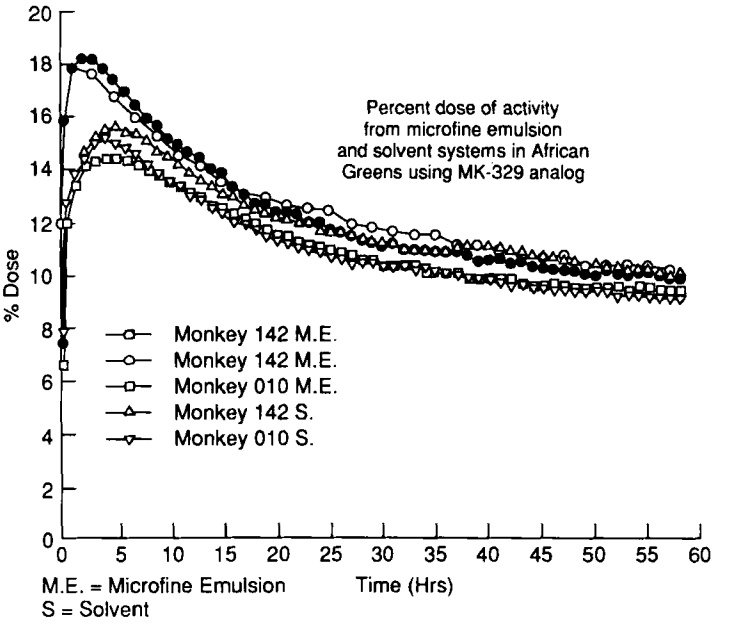

FIGURE 11

Distribution of [^{123}I]L-366,012 In Liver and Gallbladder in African Green Monkeys

component at this site had been previously established. After consideration of a number of iodinated analogs of MK-329, one was chosen whose physical characteristics resembled the parent compound.

Results using the ^{123}I labeled analog of MK-329 are presented in Figure 11. The percent dose uptake as measured by scintigraphy showed 14-18% in the liver/gallbladder region an amount also obtained using a soluble formulation of polyethylene glycol. Since liver uptake was the same with each formulation, it can be concluded that the microfine emulsion did not alter the liver uptake of the drug. Further experiments were carried out to confirm whether this iodinated analogue was useful in evaluating the microfine emulsion. To do this

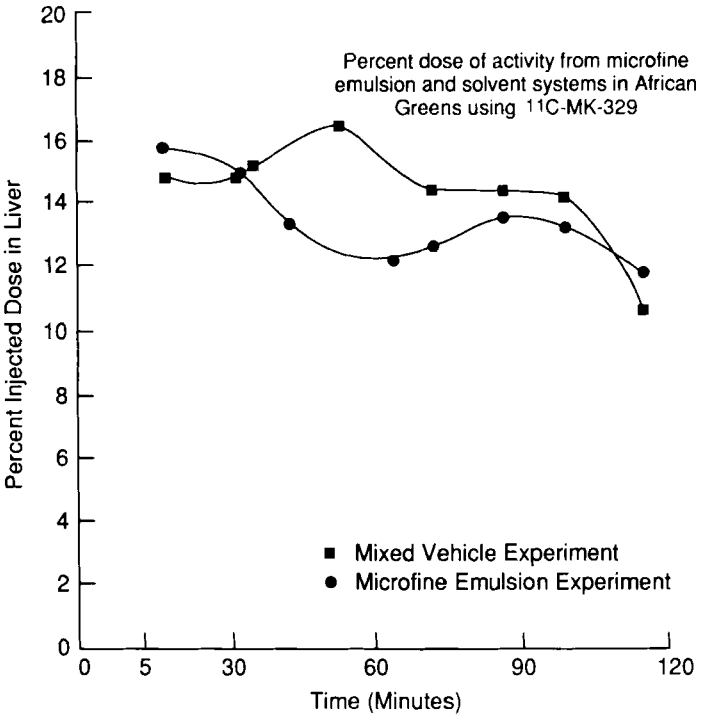

FIGURE 12

Distribution of [^{11}C]MK-329 In Liver and Gallbladder in African Green Monkeys

the parent compound was labeled with ^{11}C and imaged on a planar camera with a high energy collimator. While image quality is somewhat compromised since the collimator is not optimized for the 511 Kev energy adequate images of the area of interest could be obtained. This technique of combining positron emitters with planar imaging allows for regional or whole body views of larger organ systems. The results as seen in Figure 12 are comparable to the ^{123}I results and confirm that analogs can be used in select cases.

As illustrated in this brief review of drug delivery techniques there are numerous approaches to aid in defining delivery, release, and absorption of

compounds. Beginning with conventional radiotracers, progressing to neutron activated compounds, and finally to compounds radiolabeled with halogens or positron emitters one can chose the approach which will provide the information needed in the most reasonable way. As long as adequate safety assessment is done on the compounds all of the techniques are translatable to man, however, it is often advantageous during the developmental stages of a device to perform the experiments in animals. Scintigraphy allows one to do this in a more physiological state and often in higher order animals which more closely mimic the characteristics of man.

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