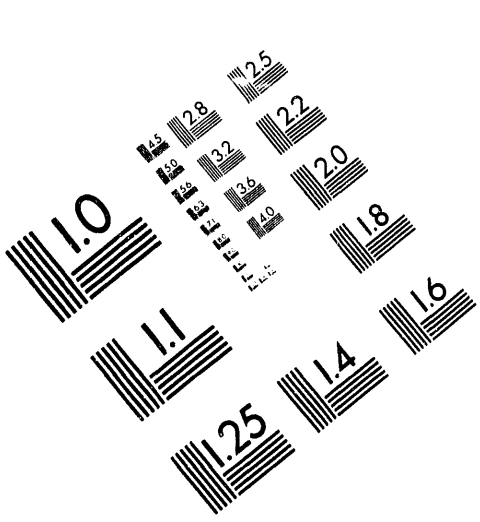

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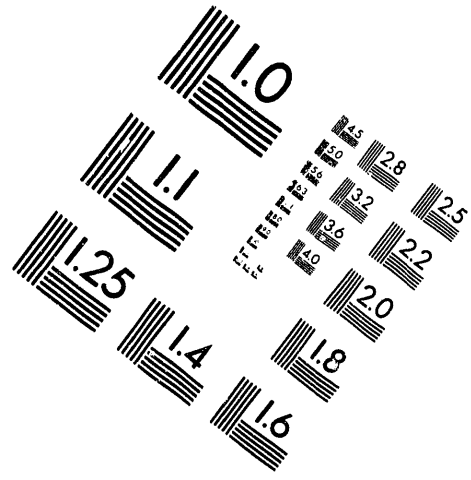

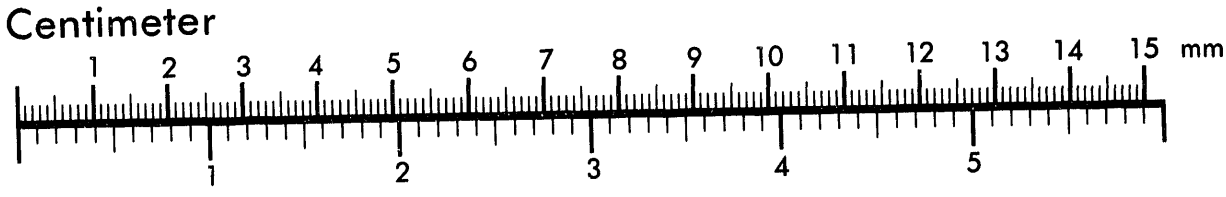

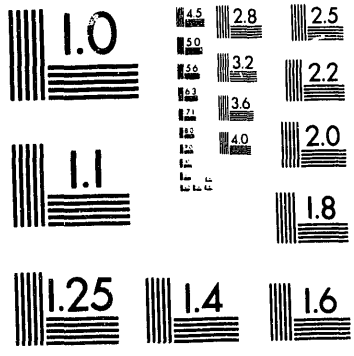

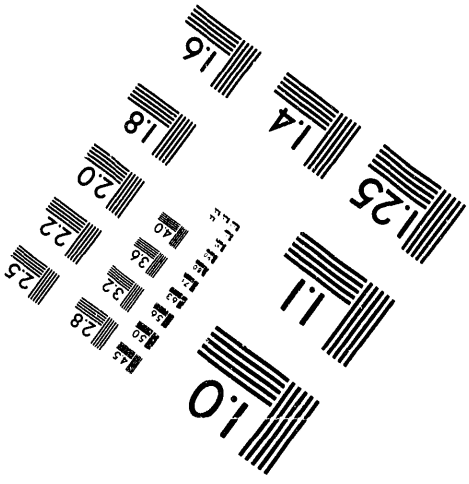

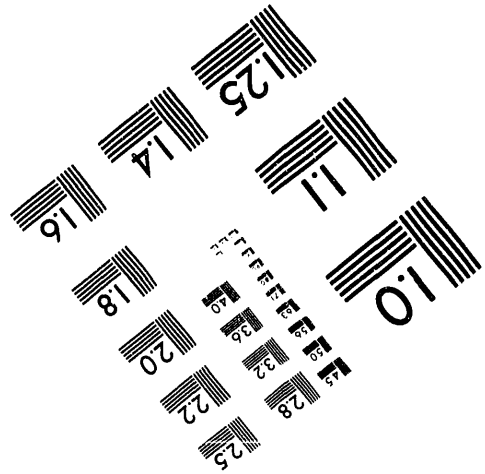

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Conf-9302119--1

BNL-48712

For, "Proceedings of the Third International Symposium on Radioiodinated Free Fatty Acids," Kyoto, Japan, February 10-11, 1993.

The Development of Iodine-123-Methyl-Branched Fatty Acids and Their Applications in Nuclear Cardiology

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Research at ORNL supported by the Office of Health and Environmental Research (OHER), U.S. Department of Energy (DOE), under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc., and at BNL under contract DE-AC02-76CH00016. The authors (FFK and JK) also thank the North Atlantic Treaty Organization (NATO) Collaborative Research Grants Program (CRG 900966) and the Deutsche Forschungs Gemeinschaft (Kr 948/1+2-1) (JK) for partial support for these studies.

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ABSTRACT

Continued interest in the use of iodine-123-labeled fatty acids for myocardial imaging results from observations from a variety of studies that in many types of cardiac disease, regional fatty acid myocardial uptake patterns are often different than regional distribution of flow tracers. These differences may reflect alterations in important parameters of metabolism which can be useful for patient management or therapeutic strategy decision making. In addition, use of iodine-123-labeled fatty acid distribution may represent a unique metabolic probe to relate some aspects of the metabolism of these substrates with the regional viability of cardiac tissue. The use of such viability markers could provide important prognostic information on myocardial salvage, helping to identify patients for revascularization or angioplasty. Clinical studies are currently in progress with the iodine-123-labeled 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP) fatty acid analogue at several institutions. The goals of this paper are to discuss development of the concept of metabolic trapping of fatty acids, to briefly review development and evaluation of various radioiodinated methyl-branched fatty acids and to discuss recent patient studies with iodine-123 (BMIPP) using single photon emission computerized tomography (SPECT).

INTRODUCTION

The high incidence of coronary heart disease (CAD), cardiomyopathies and other cardiac diseases requires the availability of effective and readily available diagnostic tools. The development of thallium-201 and other perfusion tracers for single photon and positron applications represents an important achievement in nuclear cardiology. More recently, the significant success in developing perfusion tracers radiolabeled with technetium-99m has made these diagnostic agents available on a 24 hour basis. Although the availability of perfusion tracers is important, the high false positive findings with thallium-201 and technetium-99m "Sestamibi" (MIBI) in the identification of threatened,

viable myocardium, requires the availability of complementary markers for identifying and more accurately assessing the presence of salvageable tissue.

As early as 1965, the potential of using iodine-123-labeled fatty acids for cardiac imaging was first demonstrated in human studies with iodine-131-labeled "Iodooleic acid".[1] Although beyond the scope for detailed discussion in this paper, there are reports of several attempts to develop technetium-99m-labeled fatty acids for cardiac imaging. Technetium-99m obviously would be the best candidate for radiolabeling fatty acids for SPECT from a cost standpoint and because of 24 hour availability from a $^{99}Mo/^{99m}Tc$ generator. Work reported thus far has demonstrated that the relatively complex and large chelating groups required for attachment of this radioisotope to fatty acid analogues, however, result in the formation of molecules which are evidently not recognized as fatty acids and thus have very low myocardial extraction. Iodine-123 appears to be the radioisotope of choice, and for this reason research in the SPECT area has focussed on the development of iodine-123-labeled methyl-branched fatty acids. Although the availability and costs of high purity iodine-123 have been discussed for many years, only within the last few years have a variety of new improved iodine-123-labeled agents become available for routine use. These agents cannot be radiolabeled with technetium-99m because of the issues discussed above. High purity iodine-123 is routinely available by cyclotron production by the $^{124}Xe(p,n)^{123}I$ route, for example, and the costs are evidently decreasing as wider availability increases.

Since the early work by Evans and co-workers, many investigators have made important contributions in the evolution of iodine-123-labeled fatty acids. The development of structurally-modified fatty acids became of interest because of the relatively long acquisition periods required for SPECT. The significant time required by early generation single- or dual-head SPECT systems for data acquisition (e.g. 15-30 minutes) requires minimal redistribution during the acquisition period to ensure accurate evaluation of the regional fatty acid distribution pattern after re-construction.

Extensive research has thus focussed on the evaluation of structural modifications which can be introduced into the fatty acid chain and would not interfere with myocardial extraction, but would inhibit the subsequent ß-oxidative catabolism which normally results in rapid myocardial clearance. An important requirement is, of course, that any structural changes introduced into these "modified" fatty acids will be compatible with myocyte recognition of the analogues and subsequent extraction of the analogues following intravenous administration. Several recent publications have reviewed the development and use of radioiodinated fatty acids, and include the Proceedings of the First[2] and Second[3] Workshop on Radiolabeled Free Fatty Acids and a recent text.[4]

THE DEVELOPMENT OF STRUCTURALLY-MODIFIED FATTY ACIDS

The increased myocardial retention required for SPECT imaging, especially with the early camera systems, resulted in the development of the concept of "metabolic trapping" of fatty acids resulting in their concentrating in the myocardium as a means of overcoming rapid myocardial washout. Early work at the Oak Ridge National Laboratory (ORNL) focussed on the synthesis and screening of unique fatty acid analogues in which the divalent tellurium heteroatom (Te) was introduced into the fatty acid chain (Figure 1). These studies represented the first demonstration that such a drastic modification could be made without interfering with the myocardial extraction of these fatty acids.[5,6] Incorporation of the radioactive tellurium-123m heteroatom into the fatty acid chain was the first approach to the development of a modified fatty acid in which a structural perturbation was introduced to inhibit or interfere with ß-oxidation, and thus prolong the myocardial residence time of the radiolabeled agent following flow-dependent myocardial extraction.[5] The first such analogue investigated was 9-[^{123m}Te]-telluraheptadecanoic acid (9-THDA).[5,7] Subsequently, a number of tellurium-123m-labeled analogues were synthesized, and this strategy resulted in a dramatic alteration

of the myocardial clearance kinetics as was demonstrated in small laboratory animals and canine models.[5,7,8,9] These studies demonstrated that the modified fatty acids were extracted similar to natural fatty acids, and as expected, the total chain length was important.[10] In addition, extraction of 9-THDA was shown to be flow-dependent, as demonstrated in an open-chest canine model.[8]

Insert Figure 1 Here

Tellurium-123m is reactor-produced, and the long 119 day half-life and low specific activity (apprx. 1 mCi/mg ^{122}Te at 2×10^{15} neutrons/cm^2/sec) are unfavorable properties, indicating the need for an alternative strategy. In order to use the concept of "trapping" of Te fatty acids in conjunction with the advantageous properties of iodine-123 (Figure 1), the concept of "metabolic trapping" was further extended to the synthesis and evaluation of "bifunctionalized" analogues. In these analogues the non-radioactive tellurium heteroatom was inserted within the fatty acid chain to increase myocardial retention, and the radioiodine was then stabilized on the fatty acid terminus by attachment as a *para*-iodophenyl group[11-12] or a *trans* vinyl iodide.[13-16] Such a strategy worked well, and the radioiodinated tellurium-substituted analogues behaved *in vivo* in the expected manner with rapid, high myocardial extraction and prolonged retention. Subsequent structure-activity studies clearly demonstrated that both total chain length and position of the heteroatom were important structural features affecting myocardial extraction and clearance kinetics.[17,18] The development of these analogues thus represented an important foundation for further development of the concept of metabolic blocking using radioiodinated fatty acids for cardiac imaging. The tellurium analogues easily decompose, however, and care is required in their handling because of the chemical susceptibility of tellurium to oxidation.[19] These undesirable properties were expected to preclude their use in humans, and a new approach was therefore required which would provide iodine-123-labeled

fatty acids which could be routinely used in the clinic.

The early studies with the tellurium fatty acid analogues were in drastic contrast to the view generally accepted by many investigators in the 1970's and early 1980's that the measurement of myocardial washout of free radioiodide from straight-chain radioiodinated iodoalkyl-substituted fatty acids was the only way that some aspect of "metabolism" could be measured.[20] We had developed the concept of heteroatom insertion in the alkyl chain of the modified fatty acids in order to inhibit "metabolism" for SPECT imaging. The concept of introduction of a heteroatom within the fatty acid chain as a means of inhibiting metabolism has now gained acceptance in the PET area, where an interesting new fluorine-18-labeled fatty acid has been developed in which the divalent sulfur heteroatom has been inserted within the fatty acid chain[21] based on earlier studies with Se-75-substituded fatty acids.[22]

METHYL-BRANCHING AS AN APPROACH TO INHIBIT ß-OXIDATION AND PROLONG MYOCARDIAL FATTY ACID RETENTION FOR SPECT

The tellurium-substituted fatty acids thus played an important role in demonstrating that rather unusual structural modifications could be introduced into the fatty acid chain without decreasing myocardial targeting and high extraction. Subsequent studies for PET applications demonstrated that methyl-branching in the ß (3)-position was also successful in significantly delaying the rapid myocardial clearance of straight chain fatty acids from normoxic myocardium.[23] This approach was based on the expected inhibition of ß-oxidation by the presence of a methyl group in the ß-position These results are consistent with earlier, unrelated studies which demonstrated that 3,3-dimethylphenylmyristic acid was not oxidized by liver homogenates.[24] The concept of using methyl-branching was further extended by the preparation of the racemic 3-methylheptadecanoic fatty acid

radiolabeled in the carboxyl group with carbon-11.[25-26] The methyl-branched analogue showed considerably longer myocardial retention compared with the linear heptadecanoic acid straight-chain analogue, although prolonged retention would not be expected to be so important with PET because of the rapid acquisition capability of this technology. The concept of increasing myocardial retention by metabolic blocking is important for SPECT, however, because of the relatively long acquisition periods required, especially with the earlier SPECT cameras.

Insert Figure 2 Here

The strategy of metabolic trapping was thus further extended by the preparation of radioiodinated ß-methyl-branched fatty acids (Figure 2). Development of the 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid analogue (BMIPP),[27-29] was chosen at ORNL because the linear structure of 15-(p-iodophenyl)pentadecanoic acid (IPPA) developed by Machulla and co-workers[30] (Figure 1) had an optimal chain length for myocardial extraction. In addition, stabilization of radioiodide was readily accomplished by attachment to the *para*-position of the terminal phenyl ring.[30] In parallel studies, the group at the Massachusetts General Hospital (MGH) synthesized and evaluated a similar analogue which had one less carbon in the alkyl chain, 14-(p-iodophenyl)-3-R,S-methyltetradecanoic acid (BMIPT).[31-32] A variety of well-established chemical methods which are easily adapted to "kit" radiolabeling are available for *para*-radioiodination. These include thallation-iodide displacement, copper-assisted iodide exchange with the unlabeled iodinated substrate, and the triazene decomposition reaction. It should be pointed out that high specific activity is not required, since the radioiodinated fatty acid analogues are diluted by a large plasma pool of free fatty acids, and are handled by the myocardium in an analogous manner. A 3-(R,S)-methyl analogue in the terminal iodovinyl-substituted series, 19-iodo-3-R,S-methyl-18-octadecenoic acid (Figure 2), was also prepared

and evaluated. This analogue also exhibits prolonged myocardial retention in comparison to a linear, straight-chain analogue, and is stored by incorporation into triglycerides.[27,33-37] The corresponding 3,3-dimethyl analogue (Figure 3), shows increased myocardial retention in comparison with the ß-monomethyl analogue, a difference similar to the relative myocardial retention of BMIPP and DMIPP described earlier.[34-36] The iodovinyl analogues are prepared by various electrophilic iododemetallation reactions, usually by reaction of $^{*}I^{+}$ with the requisite vinyl trialkylstannyl substrates.

In the iodoakyl-substituted series, one example evaluated is 17-iodo-3-R,S-methylheptadecanoic acid analogue.[33] Fagret and colleagues evaluated the time-activity curves of the afflux of radioactivity from rat hearts perfused by the Langendorff technique following bolus administration of radioiodinated fatty acids into the in-flow. The α-R,S-monomethyl-, α,α-dimethyl and ß-R,S-monomethyl- and ß,ß-dimethyl analogues of 16-iodohexadecanoic acid were also evaluated[39-40] These studies demonstrated that the ß-dimethyl analogue exhibited the longest myocardial retention. Studies evaluated the global myocardial uptake and clearance kinetics of these analogues in both mice and dogs, demonstrating that the 16-iodo-3-R,S-methylhexanoic acid analogue had the longest retention in comparison to the other analogues and was identified as a candidate for human studies.[41]

To obtain a greater understanding of the effects of methyl-branching on metabolism, more recent studies have evaluated the metabolism of the methyl-branched analogues discussed above in primary cultures of isolated rat hepatocytes which compared the metabolism of the various [I-125]-labeled analogues with [1-^{14}C]-palmitic acid.[42] While all of the analogues exhibited significant deiodination, the monomethyl-branched analogues exhibited greater esterification in triglycerides than the corresponding dimethyl analogues. Other studies have pursued evaluation of the influence of the methyl group in BMIPP on myocardial metabolism in isolated Langendorff-perfused rat hearts.[43-45]

These studies demonstrated that cellular accumulation of radioactivity involves accumulation of free BMIPP with the minimal incorporation into the triglyceride pool. Other studies in rats *in vivo*[46-47] and isolated Langendorff-perfused rat hearts have in contrast demonstrated incorporation of BMIPP into triglyceride storage products correlating with the observed myocardial retention.[47-52] It is unclear why the data of Fagret and Humbert, et al., are in direct contrast to other data which demonstrated significant incorporation of radioiodinated BMIPP into the triglyceride pool both *in vivo* and in isolated rat hearts.

Insert Figure 3 Here

EXAMPLES OF PRECLINICAL STUDIES ASSESSING THE BIOLOGICAL PROPERTIES OF RADIOIODINATED BMIPP

A number of animal studies with radioiodinated BMIPP which evaluated the regional myocardial distribution of this tracer in various cardiac disease models have paved the way for subsequent human studies (*vide infra*). One of the most important early observations was the demonstration of a discordance between a flow tracer (thallium-201) and methyl-branched fatty acid distribution in the free wall of the left ventricle and septal regions of hearts from rat and hamster models with hypertrophic and cardiomypathic heart disease.[53-57] An example of this discordance in regional myocardial distribution between flow and fatty acid metabolic marker distribution, is illustrated in the autoradiographic studies (ARG) shown in Figure 4. In addition, radioiodinated BMIPP has been more recently shown to be a useful tool to evaluate cocaine-induced regional myocardial metabolic changes in hypertensive rats by comparison of regional perfusion of thallium-

201 with differences in 2-deoxyglucose (2-DG) and radioiodinated BMIPP uptake.[58] While global perfusion is increased with cocaine, 2-DG uptake decreases with a concomitant increase in BMIPP uptake. These studies may provide insight into sudden cardiac death which is evidently often encountered with high risk hypertensive cocaine abusers.

Insert Figure 4 Here

In addition to studies with radioiodinated BMIPP and BMIPT agents, a variety of other methyl-branched fatty acids have been reported. In the iodophenyl series another key example is the geminal 3,3-dimethyl analogue of BMIPP, 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid, (DMIPP, Figure 2).[38] Since BMIPP exhibits slow myocardial washout it was speculated that introduction of two methyl groups would be expected to completely inhibit ß-oxidation.[36-38,45-46,59-60] Radioiodinated DMIPP exhibits significantly longer retention than BMIPP in rats (Figure 3),[38,45-46] confirmed in isolated perfused swine hearts,[59-60] and in a canine model *in vivo*.[49] DMIPP is deposited in myocardial triglyceride lipid stores with a "frozen" distribution pattern with essentially complete retention for several hours after administration. Figure 4 shows the time-activity curves of averaged data from IHDA, IPPA and DMIPP in normal flow myocardial biopsy specimens after simultaneously IV injection in six dogs.[61] Total myocardial activity and the fractional activity of the aqueous phase (containing oxidation products), l-phospho-lipids,l-triacylglycerols and unmetabolized IFA are expressed as dpm/mg tissue per millicurie injection dose. The data show that uptake of DMIPP is significantly lower compared to IHDA and IPPA. The clearance rate of DMIPP was prolonged with a halftime value of 287 min versus 11.2 min for IHDA and 13.5 min for IPPA. As found in earlier rat studies, DMIPP was slowly incorporated into triacylglycerols. Further studies with DMIPP have not been pursued, apparently due to the interest and success in use of [I-123]-BMIPP. Other examples of

methyl-branched Iodophenyl-substituted analogues include the racemic 9-methyl analogue of BMIPP[62-63] and BMIPT (*vide infra*).

Insert Figure 5 Here

EFFECTS OF STRUCTURE ON THE ON MYOCARDIAL KINETICS AND METABOLISM OF METHYL-BRANCHED FATTY ACID ANALOGUES

We chose to develop BMIPP as the racemic 3-methyl-branched analogue of IPPA, since studies with radioiodinated IPPA had demonstrated that it had an optimal chain length and that the terminal p-iodophenyl moiety did not interfere with myocardial specificity.[30] A dramatic illustration of the effects of 3-methyl-branching on myocardial retention in rat hearts *in vivo* is shown in Figure 6. The tetradecanoic acid analogue, 14-(p-iodophenyl)-3-R,S-methyltetratdecanoic acid (BMIPT, Figure 2), was also developed and has a carbon chain with one less carbon than BMIPP.[31-32,63-64] The dramatic unexpected effects which an apparently simple structural change can have on target organ specificity was demonstrated by comparative studies which showed that the myocardial extraction of the 15-carbon chain length BMIPP analogue was much higher (2:1) than the 14-carbon analogue.[31] and BMIPP has therefore evolved as the agent of choice in this methyl-branched series.

Insert Figure 6 Here

Introduction of the 3-methyl group in BMIPP was expected on theoretical grounds to inhibit ß-oxidation and essentially result in irreversible myocardial retention as shown in Figure 2. As

illustrated by *in vivo* studies (Figure 2) in small laboratory animals (Figure 5) and also humans,[36,65] the myocardial washout is considerably delayed by this structural alteration, although washout is still observed. In an attempt to isolate and characterize the chemical species which washes out, studies were initiated with the non-working Langendorff-perfused rat heart, which represented a convenient analytical system to analyze the radioactivity present in the outflow tract.[47,50-51] These studies demonstrated that the majority of the radioactive species in the outflow represented an unidentified polar metabolite (Figure 7). The same metabolite has more recently been identified in the outflow of the more physiological working isolated rat heart system,[49] and in analysis of plasma samples from humans following intravenous administration of iodine-123-labeled BMIPP.[36,65-67] These studies with the isolated rat heart system have also demonstrated that "back diffusion" of the unmetabolized BMIPP fatty acid substrate is a component of myocardial washout.[47-52] These results are similar with the earlier studies which demonstrated that back diffusion was significant in the washout of radiolabeled palmitate from ischemic zones in a canine model.[68]

Other detailed studies have used high performance liquid chromatography (HPLC) to examine the affects of structural modifications on the incorporation of various radioiodinated fatty acids into the complex phospholipids of rat and dog hearts. While IPPA is preferentially incorporated into lecithin (phosphotidylcholine) in rat hearts, BMIPP is primarily found in the cephalin (phosphotidylehtanolamine) fraction.[69-70] More recent triple-label studies using thin-layer chromatographic analysis have evaluated the incorporation of IHA, IPPA and DMIPP into the complex lipids of dog hearts *in vivo*, where a biopsy punch technique was used to obtain myocardial samples. While IHA is primarily incorporated into phosphatidyinositol and phosphatidylcholine, IPPA was found in the phosphatidylcholine fraction and DMIPP exhibited only very low incorporation into the phospholipid fraction.[61]

Although the dimethyl DMIPP analogue exhibits longer myocardial retention than BMIPP (See Figure 5; Ref. 36 and 71), radioiodinated BMIPP is currently the agent of choice in the iodophenyl series, since its clearance kinetics represent a compromise between having enough time for evaluation of regional distribution by SPECT, but exhibiting clearance kinetics which can be measured by successive SPECT acquisitions. Iodine-123-BMIPP may thus offer the first unique opportunity to construct regional time activity curves from successive SPECT studies (*vide infra*).

Several studies evaluated the properties of radioiodinated BMIPP in canine models.[72-74] More recently, the uptake and clearance of [I-123]-BMIPP has also been studied by planar imaging in a canine occlusion-reperfusion model.[75] In this protocol sequential gamma camera images obtained following intravenous administration of thallium-201 and [I-123]-BMIPP were evaluated, and the hearts were also excised and then imaged. Two dogs served as a control, one group of eight dogs had a 6 hour occlusion by ligation of the left anterior descending coronary artery before reflow (chronic), while 2 dogs had a 3 hour occlusion followed by one hour reperfusion (acute). While all dogs with the longer occlusion showed persistent defects with BMIPP and thallium-201, five of the dogs (80 %) in the second group with a much shorter occlusion period showed a mismatch of BMIPP/thallium uptake with greater uptake of BMIPP than thallium-201.

Insert Figure 7 Here

EXAMPLES OF CLINICAL APPLICATIONS OF IODINE-123-BMIPP

As discussed by several groups in these "Proceedings", there are currently various clinical protocols which are being pursued for patient use with [I-123]-BMIPP. Excellent images of the left ventricular myocardium are obtained with SPECT imaging after injection with as low as 3-5 mCi of

[I-123]-BMIPP. The first clinical studies with [I-123]-BMIPP by Dudczack and co-workers which demonstrated that this agent exhibited the expected prolonged myocardial retention and provided excellent delineation of the myocardium by planar imaging.[36,65]

SPECT studies have also been reported which include evaluation of [I-123]-BMIPP in patients with hypertrophic cardiomyopathy.[76] In recent studies fourteen patients with left ventricular hypertrophy were evaluated at rest following administration of [I-123]-BMIPP and SPECT studies conducted 20 minutes and 3 hours later. Studies were also conducted in the patients within one week with thallium-201. Quantitative analysis of the regional uptake and clearance of both BMIPP and thallium-201 demonstrated that the regional distribution of BMIPP was more heterogeneous than that observed with thallium-201. Important relative differences included lower uptake of BMIPP in the anteroseptal wall than in the posterolateral wall. Although thallium-201 uptake was normal or increased in the anteroseptal wall, BMIPP exhibited both decreased uptake and increased clearance in these same regions. In contrast to regions with only mild hypertrophy, a general finding was that thickened wall segments showed lower BMIPP uptake and faster clearance.

An evaluation of regional myocardial uptake and clearance of [I-123]-BMIPP in patients with myocardial infarction has also recently been reported.[77] This study protocol consisted of SPECT imaging at rest with [I-123]-BMIPP and thallium-201 in four normal controls and 28 patients with myocardial infarction. Contrast ventriculography of each patient also provided an opportunity to correlate tracer uptake and washout with regional wall motion abnormalities. The SPECT studies were obtained with each tracer independently within one week of each other. Image analysis consisted of a four-point grading system of the uptake of tracer in seven segments of the left ventricular myocardium with quantitative analysis by generation of time-activity curves and creation and analysis of bull's-eye polar maps. An important observation from these studies was decreased global myocardial uptake of BMIPP in 17/28 patients (61%) and in 49/196 myocardial segments

(25%). This mismatch was observed between BMIPP uptake and perfusion tracer distribution more often in areas which had suffered an acute myocardial infarction and areas which were supplied by revascularized compared with nonrevascularized areas. Most importantly, lower BMIPP uptake was more often observed in segments which exhibited wall motion scores lower than perfusion scores in comparison to segments showing a concordant decrease in both wall motion and perfusion scores.

Another approach currently used at the Clinic for Nuclear Medicine at the University of Bonn, Germany with [I-123]-BMIPP, utilizes a submaximal exercise to promote ischemia prior to tracer administration.[67] This protocol evolved from the "Dual SPECT" approach developed earlier by Kropp and co-workers for use with [I-123]-IPPA, in which the distribution of radioiodinated IPPA in the early SPECT (SPECT-I) represents blood flow, and fifteen minutes later a second acquisition (SPECT II) is obtained at rest.[78] Comparison of the differences in relative regional tracer concentration in myocardial segments between SPECT-I and SPECT II is defined as "metabolism".[78] For this analysis there is no actual "redistribution" of radioactivity between the early and late SPECT's in the usual sense. In contrast, the more rapid washout of radioactivity from normal, oxygenated segments, is contrasted in comparison to significantly delayed washout from ischemic segments. Relative differences can thus be used to differentiate between normal and ischemic segments which can be detected with appropriate timing of successive SPECT acquisitions.

A similar protocol with [I-123]-BMIPP has recently been studied in a group of 20 patients who were also evaluated by coronary angiography and left ventricular cineventriculography.[67] An initial SPECT-I acquisition after administration of 5 mCi [I-123]-BMIPP following sub-maximal exercise, followed by a second acquisition (SPECT-II) 3 hours later at rest. SPECT-III is then acquired at rest following a second tracer injection of 2 mCi of [I-123]-BMIPP. Short axis slices are analyzed by the Bull's eye display. In this initial patient group, 98% of infarctions could be detected as persistent

defects. The sensitivity and specificity to detect ischemia were 89% and 91%, respectively. A total of 94% of non-infarcted segments exhibited reduced or absent uptake of BMIPP in SPECTs I and II and regularly showed no major differences in activity distribution. These segments had normal BMIPP uptake after re-injection in SPECT III, demonstrating the attractive properties of BMIPP for differentiation between irreversibly damaged and ischemic, but still viable, myocardium. An examples of this protocol in a patient with a infarction is shown in Figure 8.

Insert Figure 8 Here

An additional recent example is a study protocol being conducted at the Nuclear Medicine Department at the Free University Hospital in Brussels (VUB), Belgium, involving administration of [I-123]-BMIPP at rest and comparison with the myocardial distribution of [Tc-99m]-Sestamibi (MIBI).[79] These studies are also being complemented with two-dimensional echocardiography and ECG-gated magnetic resonance imaging (MRI) to provide information on the evolution of wall motion during heart contraction. The goal is to correlate functional changes with changes in tracer distribution observed in the radionuclide studies and to determine if such differences can identify ischemic but viable myocardium. In one study, [I-123]-BMIPP (4 mCi) and [Tc-99m]-MIBI (25 mCi) were administered at a one day interval after overnight fasting. The SPECT results from 15 patients with recent myocardial infarction were compared with regional wall motion obtained with gated-MRI at rest and during low dose dobutamine infusion (10 mcg/kg/min) to identify ischemic but viable myocardium. Nine segments were defined on apical, mid-ventricular and basal slices to compare SPECT and gated-MRI data. A total of sixty segments had either abnormal BMIPP or MIBI uptake 37 of these segments (62 %) exhibited concordent BMIPP and MIBI uptake, while 23 segments (38 %) exhibited decreased BMIPP activity relative to MIBI. The wall motion of these 23 segments with

discordant BMIPP and MIBI uptake was normal in 7 segments and improved with low dose dobutamine in 11 segments. On the other hand, only 3 of the 37 segments with concordantly decreased BMIPP and MIBI uptake showed evidence of residual viability on the dobutamine MRI study. These results demonstrate that comparison of MIBI SPECT (perfusion) with BMIPP SPECT is useful in identifying viable myocardium after acute myocardial infarction.

A typical example from this study protocol is shown in Figure 9, which illustrates [I-123]-BMIPP and [Tc-99m]-MIBI SPECT studies in the same patient with a history of long term coronary artery disease. The patient was admitted to the Cardiac Care Unit with an acute myocardial infarction of the inferior wall two weeks prior to the radionuclide studies. Therapy consisted of thrombolysis with intravenous Streptokinase (1.5 million units in 30 minutes) followed by Heparin (30,000 units/day/5 days). Analysis of the short axis SPECT results illustrate that both the extent and severity of decreased BMIPP activity in the inferior wall exceed that observed with MIBI. These results suggest the presence of residual jeopardized myocardium in the peri-infarction regions after thrombolysis. Additional BMIPP/MIBI mismatching is observed in the antero-septal region near the apex.

Insert Figure 9 Here

The results of contrast ventriculography and coronary arteriography obtained in this patient two days after the radionuclide studies shown in Figure 8 demonstrated moderate hypokinesis of the inferior wall and successful recanalization of the right coronary artery, with a residual stenosis of 75%. In these studies a severe 95% stenosis of the mid portion of the left anterior descending coronary artery was also found. These results could explain the BMIPP/MIBI mismatching in the antero septal region near the apex which exhibited normal MIBI uptake and decreased BMIPP uptake

(Figure 8). Since the patient had a history of chronic angina pectoris, the chronic attacks in this territory may have induced subtle regional metabolic changes reflected by decreased BMIPP uptake (and thus decreased fatty acid utilization) which persist longer than the regional blood flow abnormalities.

FUTURE STUDIES WITH IODINE-123 METHYL-BRANCHED FATTY ACIDS

The issue of myocardial viability will continue to be an important factor in determining patient therapy and it would appear that radiolabeled agents such as [I-123]-BMIPP will be required to provide information which cannot be obtained with flow tracers. As described earlier, a number of studies have elucidated various aspects of the metabolic fate and physiological factors affecting the myocardial uptake, which include triglyceride storage and washout of radioiodinated BMIPP,[45-46,48] altered uptake in diabetic myocardium,[80] discordance between blood flow and uptake in lactate infusion,[81] the possibility of tumor detection,[82] uptake in adriamycin-induced cardiomyopathy,[83] and the effects of ATP on BMIPP uptake.[84] It is still unclear, however, how the deficits in regional BMIPP uptake observed in the SPECT cross sectional images correlate with some aspect of aberrations in the mechanism of fatty acid uptake or metabolism. Obviously, the detection of only low levels of activity in these regions reflects the absence of exogenous fatty acid extraction exhibited by low BMIPP uptake.

An aberration in contractile function in myocardial regions which have low fatty acid extraction but adequate perfusion demonstrated with flow tracers indicates that factors which are currently unknown or not well understood affect myocyte fatty acid uptake. Studies with [1-^{11}C]palmitate in dogs have clearly shown that there is a significant delay in recovery of contractile function and thus fatty acid uptake in the canine heart following reperfusion after coronary artery

occlusion.[85] If fatty acids are not being extracted from the plasma for energy production, either endogenous myocardial fatty acid stores are depleted and other energy stores are being consumed for maintenance of viability. Viability in the presence of marginal contractile function would indicate that these segments are "hibernating". Some factors which could result in significantly decreased extraction of exogenous fatty acids in viable myocardial regions may include alterations in fatty acid binding protein(s) involved in the transfer of fatty acids.[86-88]

In comparison with earlier studies of ischemic myocardium[86] myocardial segments which are viable but have decreased contraction would presumably be expected to concentrate 3-fluorodeoxglucose (3-FDG). In these regard, approaches including radioiodinated fatty acid uptake and ventricular function studies in conjunction with studies with [fluorine-18]-labeled-3-FDG would evaluate the factors which result in deceased fatty extraction in myocardial segments that have adequate perfusion but demonstrate significantly decreased contractile function hopefully will also be pursued.

Insert Figure 10 Here

Other studies which would be expected to provide additional insight from the results of animal models would include a biochemical and histological analysis of biopsy segments removed from the myocardial regions which have decreased fatty acid uptake. Since the washout of BMIPP is considerably delayed in comparison to the IPPA straight-chain analogue (Figure 4), the measurement of differences in washout between normal and ischemic myocardial segments, for instance, could be studied by construction of regional time-activity curves of normal versus abnormal regions using the new three-head SPECT systems which have a rapid acquisition capability. One can thus envision an approach similar to the use of [1-^{14}C]-palmitate studies with PET.

Therefore, continued animal studies in conjunction with ongoing patient studies would be expected to provide models for interpretation and routine clinical use of iodine-123-labeled fatty acids and their role in evaluating the significance of decreased extraction in regions with normal perfusion and decreased contraction.

ACKNOWLEDGEMENTS

Research at ORNL supported by the Office of Health and Environmental Research (OHER), U.S. Department of Energy (DOE), under contract DE-AC05-84OR21400 with the Martin Marietta Energy Systems, Inc. The authors (FFK and JK) also thank the North Atlantic Treaty Organization (NATO) Collaborative Research Grants Program (CRG 900966) and the Deutsche Forschungs Gemeinschaft (Kr 948/1 + 2 -1) for partial support (JK) for these studies.

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FIGURE LEGENDS

Figure 1. Structures of various tellurium (Te)-substituted fatty acid analogues fatty acids evaluated for myocardial imaging.

Figure 2. Mechanism of ß-oxidation and the expected inhibition of catabolism by introduction of a methyl-group in the ß (3)-position of fatty acid analogues.

Figure 3. Structures of radioiodinated methyl-branched fatty acids.

Figure 4. Comparison of "mismatch" between distribution of [I-131]-BMIPP and [T1-201]-thallous chloride in cross-sectional slices of hearts from normal and cardiomyopathic hamsters determined by autoradiography. Upper panel, normal hamsters; left, BMIPP; right, thallous chloride. Lower panel, cardiomyophatic hamsters; left, BMIPP; right, thallous chloride.

Figure 5. Time activity curves from a triple-label study illustrating the kinetics of IPPA, IHA and DMIPP in normal canine myocardium (n=6) (Sloof, et al., 1993).

Figure 6. Triple-label study comparing myocardial uptake and retention of radioactivity after simultaneous intravenous administration of a mixture of [I-131]-IPPA, [I-125]-BMIPP and [I-123]-DMIPP to fasted rats. Although global myocardial extraction is not significantly altered, the effects of methyl-substitution on myocardial retention are quite dramatic (Knapp, et al., 198?).

Figure 7. Illustration of the washout kinetics of radioiodinated components from the outflow tract from a dual-label study involving simultaneous administration of [I-125]-BMIPP and [I-131]-IPPA into the inflow of non-working Langendorff retrograde-perfused rat hearts. The curves were constructed from counting samples from the outflow. Higher values for IPPA represent greater washout. Samples from the 5 minute perfusate were analyzed by TLC (SiO_2, ether:petroleum ether:acetic acid, 70:30:1). While IPPA and its expected catabolites are observed by TLC analysis, BMIPP washout consist of both back diffusion and also significant levels of an unidentified polar metabolite (Knapp, et al., 199?).

Figure 8. Patient study at the Clinic for Nuclear Medicine at the University of Bonn, Germany, with [I-123]-BMIPP using a protocol with three successive SPECT acquisitions. SPECT I was obtained after i.v. administration of 5 mCi of [I-123]-BMIPP immediately following sub-maximal exercise and SPECT II was obtained 3 hours later at rest. SPECT III was then obtained at rest following a second administration of 2 mCi of [I-123]-BMIPP. The four slices represent apical and base regions and two mid-ventricular slices (Apex to Base, Left to Right) (Kropp,et al., 1993).

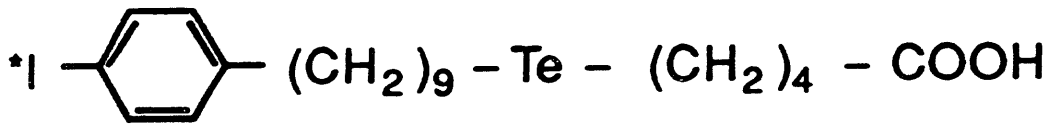
Figure 9. Example of a rest study from the Nuclear Medicine Department at the Free University Hospital in Brussels, Belgium, comparing SPECT studies obtained on different days following administration of 5 mCi of [I-123]-BMIPP and 25 mCi of [Tc-99m]-MIBI to a patient with an inferior wall infarction following thrombolysis. See text for details of this study. The four slices represent apical and base regions and two mid-ventricular slices (Apex to Base, Top to Bottom) (Franken, et al., 1993).

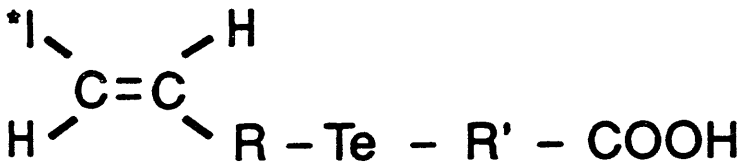
Figure 10. Binding and transport of fatty acids and their derivatives across the cytosol of myocytes. VLDL, Very low density Lipoprotein; LPL, Lipoprotein lipase; FA, Fatty acid; FABP, fatty acid binding protein; IS, Interstitial space; ACS, Acyl-CoA synthetase, dotted lines: possible interactions with the adenine-nucleotide translocator and the Na-K-ATPase, modified according to paper of Glatz, in, "News Physiol Science."

ORNL-DWG 93Z-5179

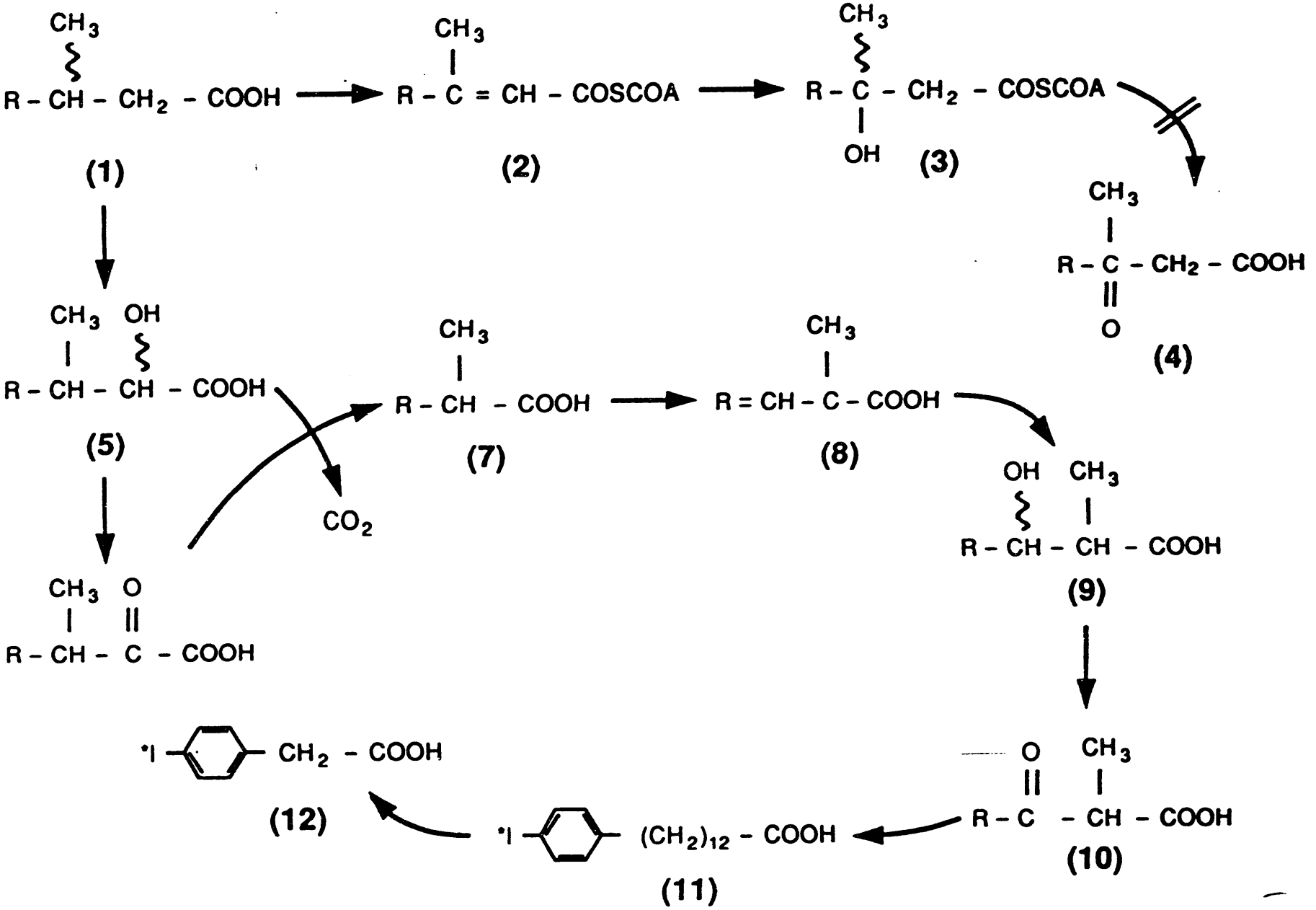
$H_3C-(CH_2)_7-{}^{123m}Te-(CH_2)_7-COOH$ **9-THDA**

$H_3C-R-{}^{123m}Te-R'-COOH$

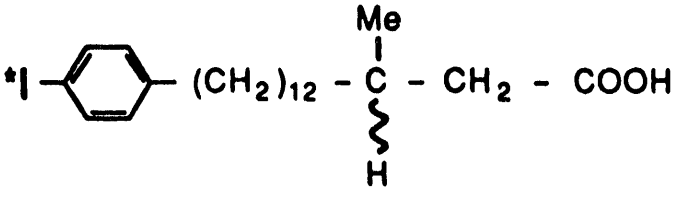




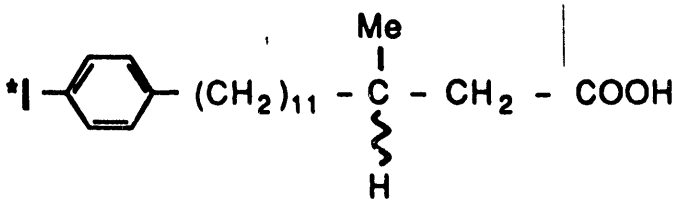
(trans)


R – CH – CH2 – COOH
(1)
R – C = CH – COSCOA
(2)
R – C – CH2 – COSCOA
OH
(3)
R – C – CH2 – COOH
O
(4)
R – CH – CH – COOH
(5)
R – CH – COOH
(7)
R = CH – C – COOH
(8)
CO2
R – CH – CH – COOH
(9)
R – C – CH – COOH
(10)
(CH2)12 – COOH
(11)
CH2 – COOH
(12)

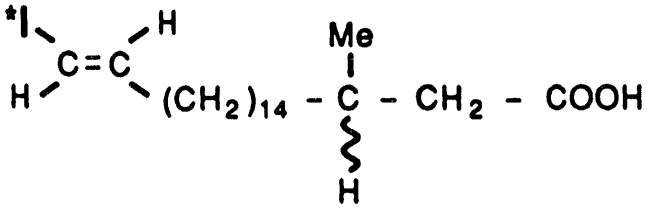
ORNL-DWG 93Z-5178



BMIPP

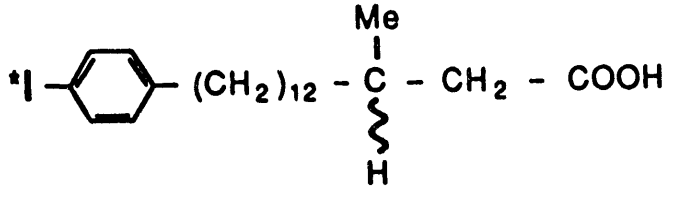


BMIPT

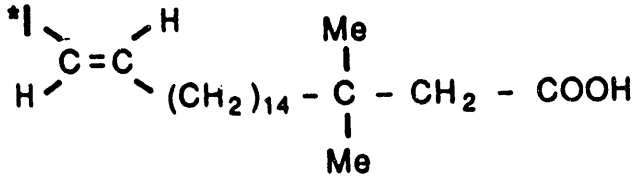


IVN

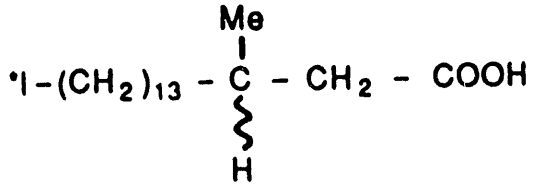
ORNL-DWG 93Z-5242

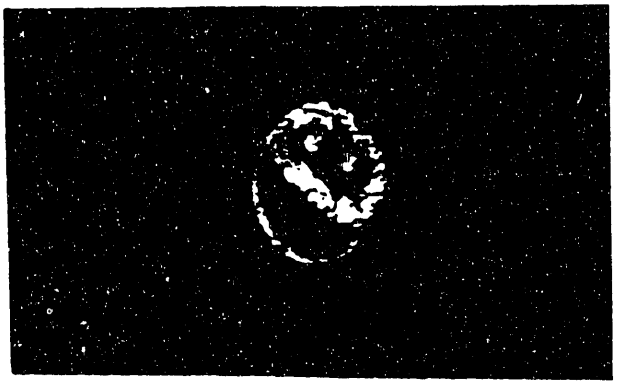


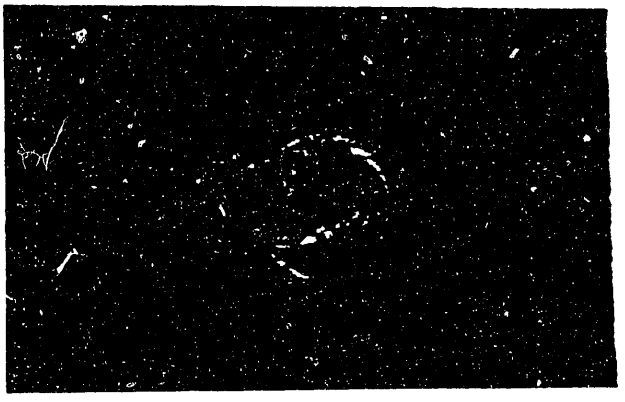
DMIPP

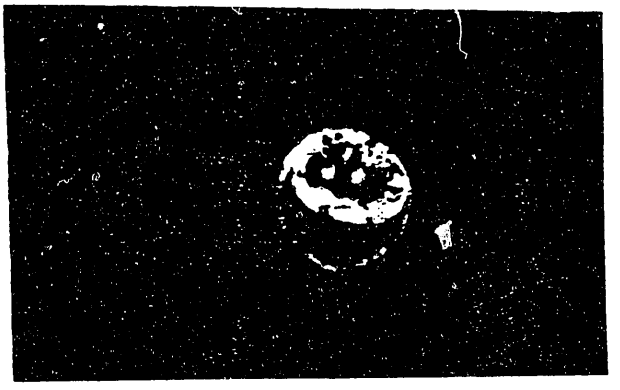


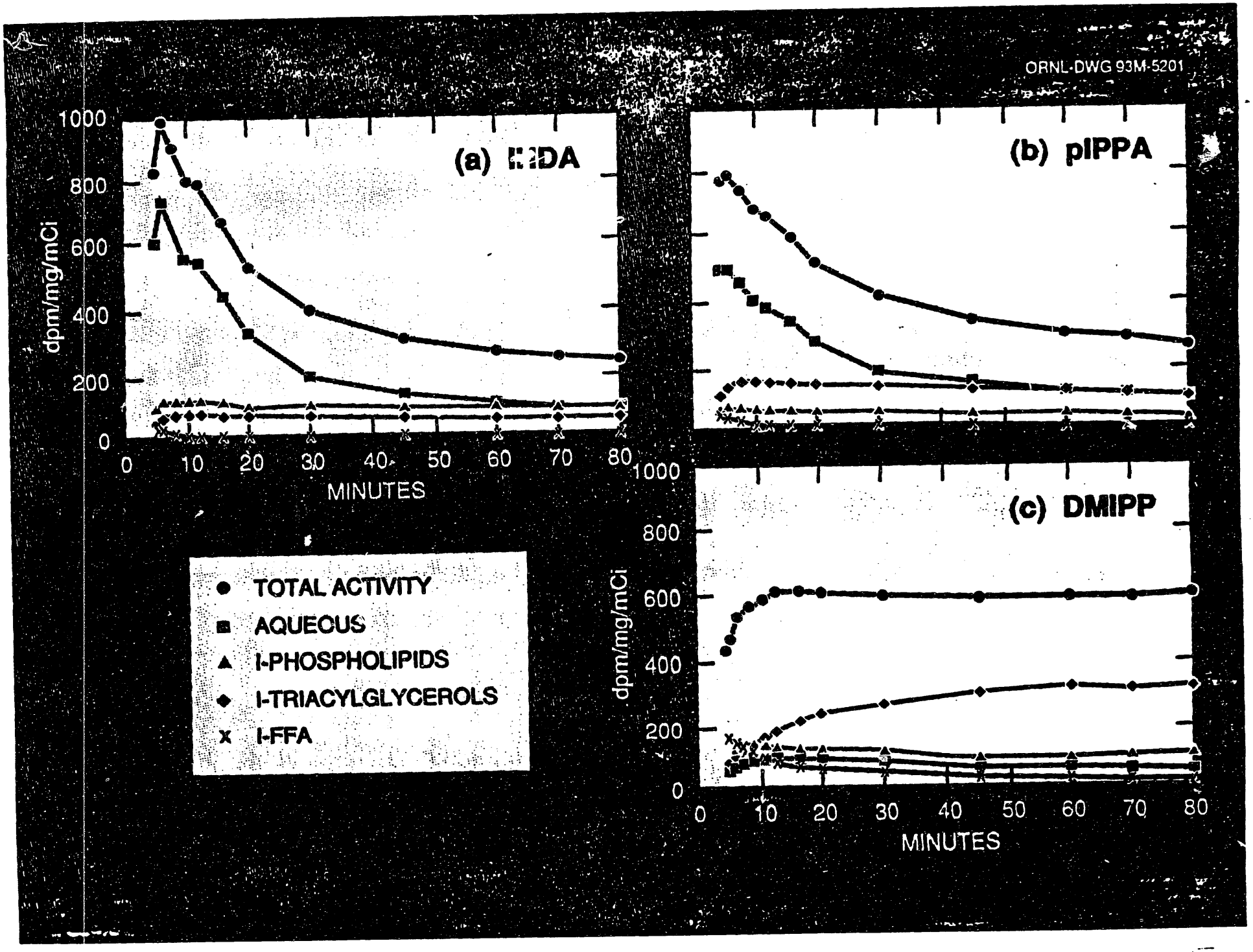
DMIVN



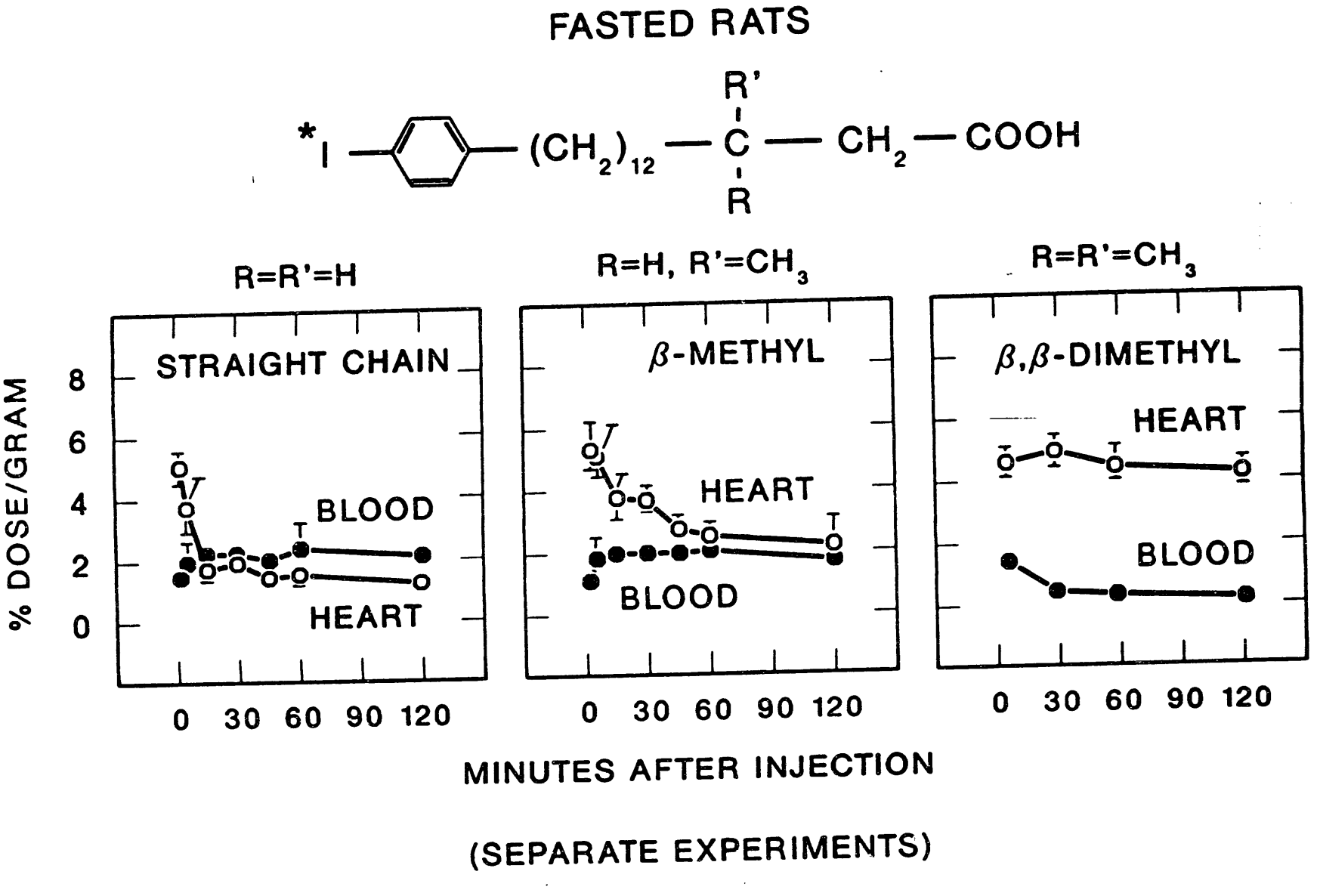


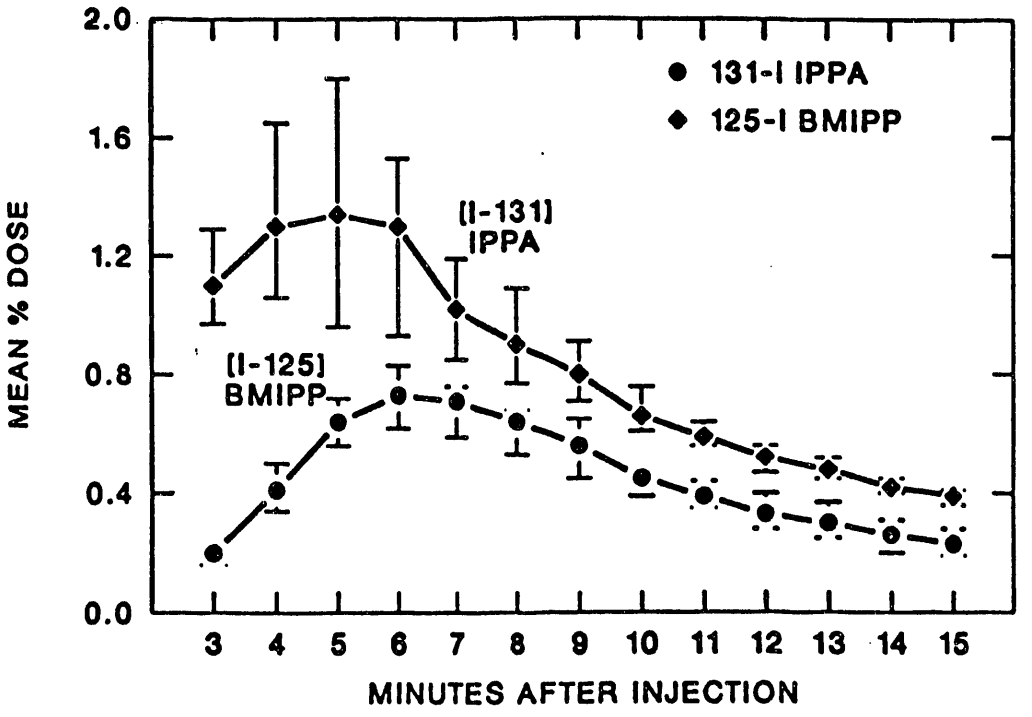


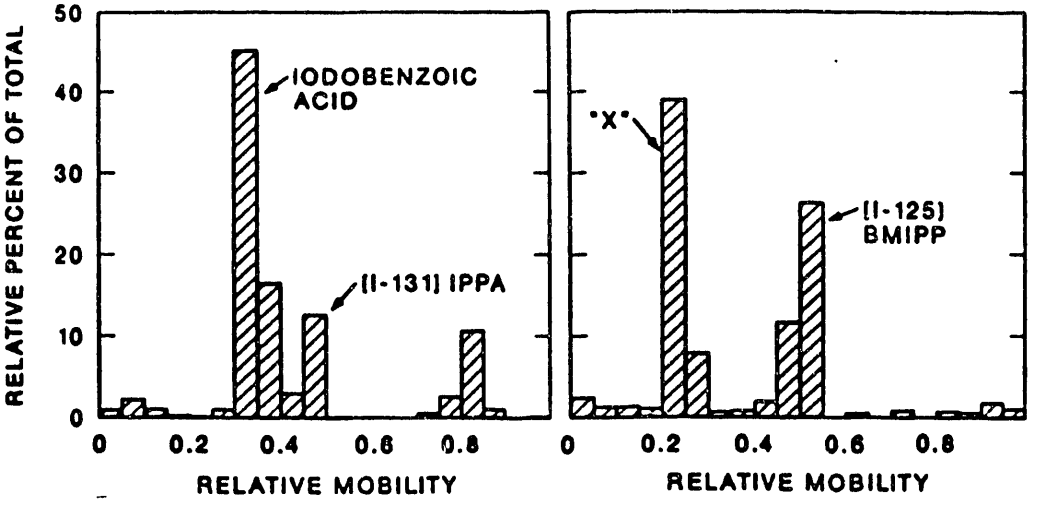


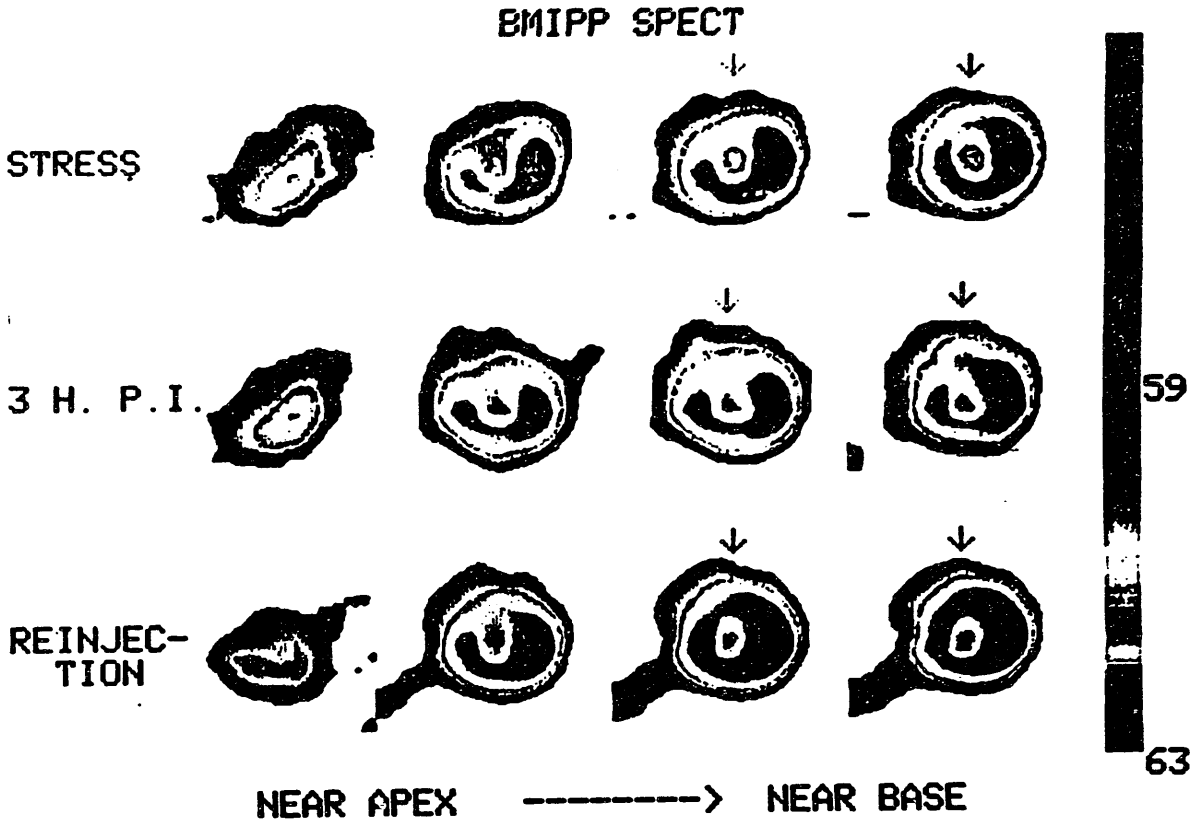

ORNL-DWG 93M-5201
(a) EDA
(b) pIPPA
(c) DMIPP
dpm/mg/mCi
MINUTES
TOTAL ACTIVITY
AQUEOUS
I-PHOSPHOLIPIDS
I-TRIACYLGLYCEROLS
I-FFA

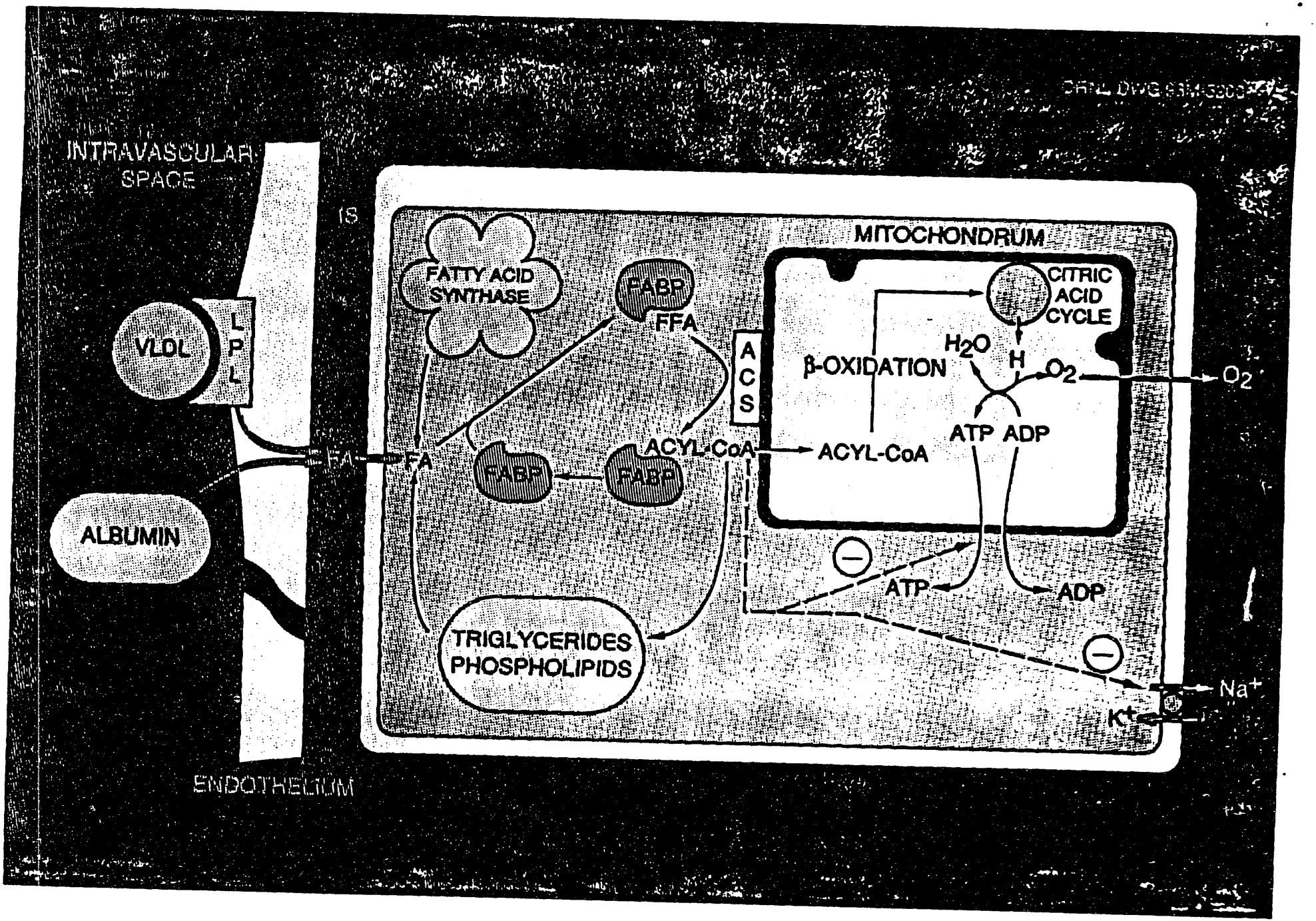
ORNL-DWG 85C-13631




2.0
1.6
1.2
0.8
0.4
0.0
MEAN % DOSE
131-I IPPA
125-I BMIPP
[I-131] IPPA
[I-125] BMIPP
3 4 5 6 7 8 9 10 11 12 13 14 15
MINUTES AFTER INJECTION


50
40
30
20
10
0
RELATIVE PERCENT OF TOTAL
IODOBENZOIC ACID
[I-131] IPPA
0 0.2 0.4 0.6 0.8
RELATIVE MOBILITY
"X"
[I-125] BMIPP
0 0.2 0.4 0.6 0.8
RELATIVE MOBILITY


BMIPP SPECT
STRESS
3 H. P.I.
REINJEC-
TION
59
63
NEAR APEX ---------> NEAR BASE


INTRAVASCULAR SPACE
VLDL
LPL
ALBUMIN
ENDOTHELIUM
IS
FATTY ACID SYNTHASE
FABP
FFA
MITOCHONDRUM
CITRIC ACID CYCLE
ACS
β-OXIDATION
H2O
H
O2
O2
ATP ADP
FA
FA
FABP
FABP
ACYL-CoA
ACYL-CoA
ATP
ADP
TRIGLYCERIDES PHOSPHOLIPIDS
Na+
K+

DATE
FILMED
8/18/93

END