PROGRESS REPORT

For Period October 1976 through June 1979

# AN INTERACTIVE RADIOPHARMACEUTICAL FACILITY BETWEEN YALE MEDICAL CENTER AND BROOKHAVEN NATIONAL LABORATORY

Prepared for

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under

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## DISCLAIMER

PROGRESS REPORT



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NOTICE

# PROGRESS REPORT

DOE Contract No. EY-76-S-02-4078 was started in October 1976 to set up an investigative radiochemical facility at the Yale Medical Center which would bridge the gap between current investigation with radionuclides at the Yale School of Medicine and the facilities in the Chemistry Department at the Brookhaven National Laboratory. To facilitate these goals, Dr. Mathew L. Thakur was recruited who joined the Yale University faculty in March of 1977.

This report briefly summarizes our research accomplishments through the end of June 1979. These can be broadly classified into three categories:

1.1 Research using Indium-111 labeled cellular blood components

1.2 Development of new radiopharmaceuticals

1.3 Interaction with Dr. Alfred Wolf and colleagues in the Chemistry Department of Brookhaven National Laboratories.

## 1.1 Research Using Indium-111 Labeled Cellular Blood Components

### 1.1a Viability, Random Migration, Chemotaxis, Bactericidal Capacity and Ultrastructure of In-111 Labeled Human Polymorphonuclear Leukocytes (PMN)

McAfee and Thakur had concluded from their in vitro survey (Part I, J. Nucl. Med. 17:480 1976 and Part II, J. Nucl. Med. 17:488 1976 ) that Indium-111 chelated to 8-hydroxyquinoline (oxine) was a suitable tracer for labeling white blood cells (PMN). Thakur and colleagues have subsequently evaluated the ability of In-111 labeled WBC to accumulate in abscesses in dogs and humans (J. Lab. Clin. Med. 89:217 1977); J. Nucl. Med. 18:1014 1977) Exp. Hematol. 5, Supplement 1, 145 1977 ). During the labeling procedure, the PMN are handled, isolated and exposed to In-111. The radionuclide penetrates through the cell membrane and binds to cytoplasmic components (Thakur et al., J. Nucl. Med. 18:1022 1977 ). During this process, a

radiation dose of several kilorads is received by each cell. Furthermore, the PMN are also exposed to ethanol and oxine. It is conceivable that any of these factors may alter the functional ability of PMN. This partial loss of functional ability resulting from the labeling procedure might not be detectable by gross in vivo results such as "abscess detection."

We therefore decided to systematically investigate the effects of In-111 labeling on viability, random migration (non-directional locomotion of cells), chemotaxis (directional locomotion of cells in chemotactic gradient), bacterial capacity, and ultrastructure of human PMN.

The work was carried out in vitro with the collaboration of the Infectious Disease Section at Yale. The in vitro study allowed us to control the desired conditions most satisfactorily. The experimental conditions and protocol are described in a preprint (J. Nucl. Med., July, 1979) attached in Section 4.1 of this report.

The result of this work indicated that the conditions of the labeling procedure performed in our laboratory do not alter either the viability, random migration, chemotaxis, bactericidal capacity, or the ultrastructure of human PMN's. Ethanol concentrations up to 80 mg (20 times the upper limit of standard labeling concentration) and 29 μCi of Indium-111-oxine per $10^6$ cells (6 times the upper limit of standard labeling concentration) showed no adverse radiation effects. Oxine was found to be the most injurious of all the labeling components. However, no damage is caused until $10^6$ cells are exposed to 10 μg of oxine, which is ten times more than the upper limit of the normal labeling oxine concentration. The mechanism by which oxine injures the cell is not fully understood. However, this work ascertained that the normal labeling procedure causes little alteration of

the cell function as measured by the classic PMN functional parameters described above.

1.1b Imaging the Inflammatory Response to Experimental Myocardial Infarction with In-111 Labeled Autologous Leukocytes: Effects of Infarction Age and Residual Regional Myocardial Blood Flow

The infiltration of polymorphonuclear leukocytes (PMN) into regions of acute myocardial infarction is a well-characterized histopathological event. Mallory et al., (Amer. Heart J. 18:647 1939 ) have demonstrated with necropsy study that in human infarctions PMN begin to infiltrate within 24 hours and continue to do so for the next 3 days. Thereafter, the response begins to decrease and by the 14th day, PMN's completely disappear from the lesion. A series of experiments were undertaken to evaluate the potential of In-111 labeled PMN as to image the inflammation from association with myocardial infarction in vivo and to study the effects of infarction age and residual regional blood flow upon the infiltration of PMN into the lesion.

The study was performed in 29 dogs subjected to acute anterior wall myocardial infarctions induced by closed chest catheter plug embolus technique described by Zaret et al. (Circulation 53:422 1976 ). Five groups of animals (n=4-6) were injected with autologous In-111 PMN's at various intervals following infarction and imaged in vivo with a gamma camera 24 hours later. Prior to sacrifice, Sr-85 labeled carbonized microspheres were injected in the left atrium, to determine relative regional myocardial blood flow. The determination of the In-111 radioactivity in the carefully dissected myocardial tissue samples from various blood flow zones permitted the quantification of PMN infiltration. The details of the protocol and results of this study can be seen in a preprint of this work, attached in Section 4.2 of this report (In press, Circulation, Aug., 1979).

Briefly, these data indicated that the inflammatory response to myocardial infarction can be imaged with In-111 PMN. All the animals injected with PMN's at 24 to 96 hours post-infarction gave positive images when imaged 24 hours after injection. At 120 hours post-infarction, however, the images were negative.

In general, PMN infiltration occurred primarily in infarct zones with residual blood flow of less than 0.6 times normal and was maximal in the lowest blood flow zones (≤0.1 times normal). The maximal epicardial infiltration (14.8 ± 3.8 times normal) occurred within the first 24 hours following infarction and the maximal endocardial infiltration (26.8 ± 4.9 times normal) occurred at 72 hours post-infarction. Thus, In-111 PMN's are a suitable agent for imaging the inflammatory response to MI in the dog model, and these data provide a justification for preliminary human trials.

1.1c Imaging Experimental Infective Endocarditis with In-111 Labeled Cellular Blood Components

The early diagnosis of infective endocarditis may be a difficult clinical problem. In nuclear medicine very little work has been directed to solving this problem, and no consistently effective radioactive agent currently exists for imaging bacterial endocarditis (BE). Our earlier work (Riba et al., Circulation 58:11 1978 --reprint attached in Section 4.3) in animals indicated that Tc-99m pyrophosphate could be used as an agent for imaging experimental BE. However, uptake in the vegetations was variable and diagnosis was difficult because of uptake in the adjacent rib cage and sternum.

The lesions of BE are vegetations consisting of bacteria, platelets and fibrin adhering to the damaged valve. Very few PMN and red blood cells are found in the lesion (Angrist et al., JAMA 183:249 1963 ). Since In-111 oxine also labels isolated platelets effectively and since labeled platelets have been shown to accumulate in

other platelet rich lesions such as the lesion of deep venous thrombosis and artherosclerosis in animals (Thakur et al., Thrombosis Research 9:345 1976 ) and in humans (Goodwin et al., J. Nucl. Med. 19:626 1978 and Davis et al., Lancet 1:1185 1978 ), we believed indium labeled platelets might be an effective tracer to detect BE.

During the second year of this contract, we evaluated the use of In-111 labeled platelets and PMN's as potential agents for imaging infective BE. Aortic valve infective endocarditis was established in New Zealand rabbits using a modified technique of Durack and Beeson (Br. J. Exp. Path. 54:142 1973 ). The details of the experimental methods can be seen in our article published in Circulation (Riba et al., 59:336 1979 ) and attached in Section 4.4 of this report.

This work demonstrated the ability of In-111 labeled platelets to detect experimental aortic valve BE in vivo. The cardiac images revealed focal areas of enhanced radioactivity uptake in the region of the aortic valve which conformed to the anatomic extent of the lesion demonstrated by gross pathology. The In-111 platelet uptake in the lesions from 17 animals averaged 240 $\pm$ 41 times greater than the radioactive uptake in equal weight of blood. In contrast, the optimal uptake in the lesion using In-111 PMN as an agent averaged only 5 $\pm$ 2 times greater than in the normal myocardium uptake and 3 $\pm$ 1 times greater than the blood radioactivity. We believe clinical trials are now warranted with the use of In-111.

1.1d Imaging Experimental Coronary Artery Thrombosis with In-111 Platelets

The role of coronary artery thrombosis in the pathogenesis of ischemic heart disease and the frequency of coronary artery thrombosis in patients with infarction and sudden death is controversial. Research in assessing the precise role of platelets in the pathogenesis of acute and chronic coronary artery disease has been limited by

the lack of suitable radioactive tracers for in vivo studies. Since In-111 has suitable imaging properties and can be incorporated into platelets without altering aggregability, we have investigated the potential of In-111 labeled autologous platelets as an agent for in vivo detection of experimental coronary arterial thrombosis.

Acute coronary artery thrombosis was established in adult mongrel dogs using a modification of the catheter electrode method of Salazar (Circ. Res. 9:1351 1961 ). Autologous platelets labeled with In-111 were used for in vivo gamma camera imaging performed at multiple intervals after platelet injection. Subsequently, the animals were sacrificed and tissue samples were obtained for quantification of radioactivity deposited in the thrombus, blood, and normal myocardium. The experimental details are included in the preprint (Circulation, In press, Oct., 1979) enclosed in Section 4.5 of this report.

In brief, we have obtained the following results.

1. All 12 dogs with acute coronary artery thrombosis, imaged within 1-2 hours of In-111 platelet administration, had positive gamma camera scintigraphs with an intense uptake in the thrombosis readily discernible from the blood pool radioactivity in the heart.

2. The radioactivity in the thrombi of these animals averaged 69 $\pm$ 10 times greater than the radioactivity in equal weight of circulating blood and was 651 $\pm$ 135 times greater than the radioactivity in equal weight of normal left ventrical myocardium.

3. There was no radioactivity uptake in 24 hour old thrombi and the in vivo scintigraphs were negative consistent with the observation that at 24 hours intact platelets are no longer recognizable since the thrombus consists primarily of fibrin.

In short, these data suggest that radiolabeled platelets have the potential to investigate the role of the coronary artery thrombosis in myocardial infarction presuming the onset of the initial pathophysiologic process is considered in relation to platelet scintigraphy.

1.2 Development of New Radiopharmaceuticals

1.2a Radioactive Arachidonic Acid (AA)

A radiopharmaceutical that would exploit the noninvasive potential of nuclear medicine to provide additional insight into cardiovascular pathophysiology is desirable. Arachidonic acid, a precursor to prostaglandins, has this potential. Berger et al., (Circulation Res. 38:566 1976 ) in this institution have demonstrated that prostaglandin (PG) E and F, potent vasoactive substances, are released during acute coronary occlusion. In addition, PG has a potential role in myocardial metabolism in that PG or its precursor (AA) may either be avidly taken up or avidly metabolized during ischemia to account for the release of PG-E and PG-F. In addition, AA as a 4-double bond, 20-carbon chain fatty acid is also a primary energy substrate for myocardium.

There are three possible gamma-emitting radionuclides that can be incorporated into AA. These are C-11 (t ½ - 20 minutes, β +), I-131 (t ½ - 8.4 days, γ-364 keV (84%))and I-123 (t ½ - 13.3 hours, γ - 159 keV (83%)).

During the past few months we have developed a technique for radioiodination of AA and begun distribution studies in dogs comparing iodinated AA to H-3 AA. Since these data have not yet been published, a detailed account is given below.

Preparation of Radioiodinated AA

Chemical purity of AA (obtained from Sigma) was established by high pressure liquid chromatographic (HPLC) techniques. Water Associates' microbond C-18 column

was used as a stationary phase and 100% Acetonitrile as a mobile phase. The elution pattern associated with impurities is shown in Figure 1.2.a-1. Quantitation of these results revealed that the AA was 95.6% pure. Chemical studies in the past (Mowny et al., J. Biol. Chem. 142:671 1942) have shown that it is difficult to maintain AA in greater than 95% purity since the double bond slowly react with traces of oxygen, ultimately producing isomers and polymers.

For iodination, 1 ml stock solutions of 0.027 M AA/ml of 50% propylene glycol in water were made in sealed vials. These were stored in the dark, at $-10^{o}$C. The purity of the stock solution of AA was checked periodically. A 100 µl aliquot of this solution is dispensed in a sealed reaction vial covered with aluminum foil and kept in an ice bath. To this is added a required volume of carrier free I-131 or I-123 iodide solution containing a desired quantity of the radioactivity. No carrier iodide is added. The mixture is acidified by adding 100 µl, 6 M HCl. The iodination is preceded by the addition of 100 µl of 0.047 M $KIO_3$ in water. The reaction mixture is incubated 15 minutes in cold. The reaction yields greater than 95% radioactive labeling of AA by the following reaction:

$$6H+ + 5{*}I^- + IO_3^- \rightarrow 3{*}I_2 + 3\,H_2O$$

The quantity of iodine atoms was maintained substantially lower than the available capacity of AA for iodination. (In theory, each molecule of AA can bind 4-8 atoms of iodine across its double bonds). Therefore, the reaction we use produces high specific activity radioactive AA which may not be saturated with iodine atoms. Furthermore, since the $I_2$ is produced instantaneously in a closed vessel, iodination is rapid. The use of propylene glycol has several advantages. It dissolves AA, which is not soluble in any of the aqueous reagents used in the reaction mixture and keeps it in solution in the presence of an

HPLC - ARACHIDONIC ACID
C-18 μ-BONDPACK COLUMN
100% $H_3CC{\equiv}N$, 2 ml / MIN

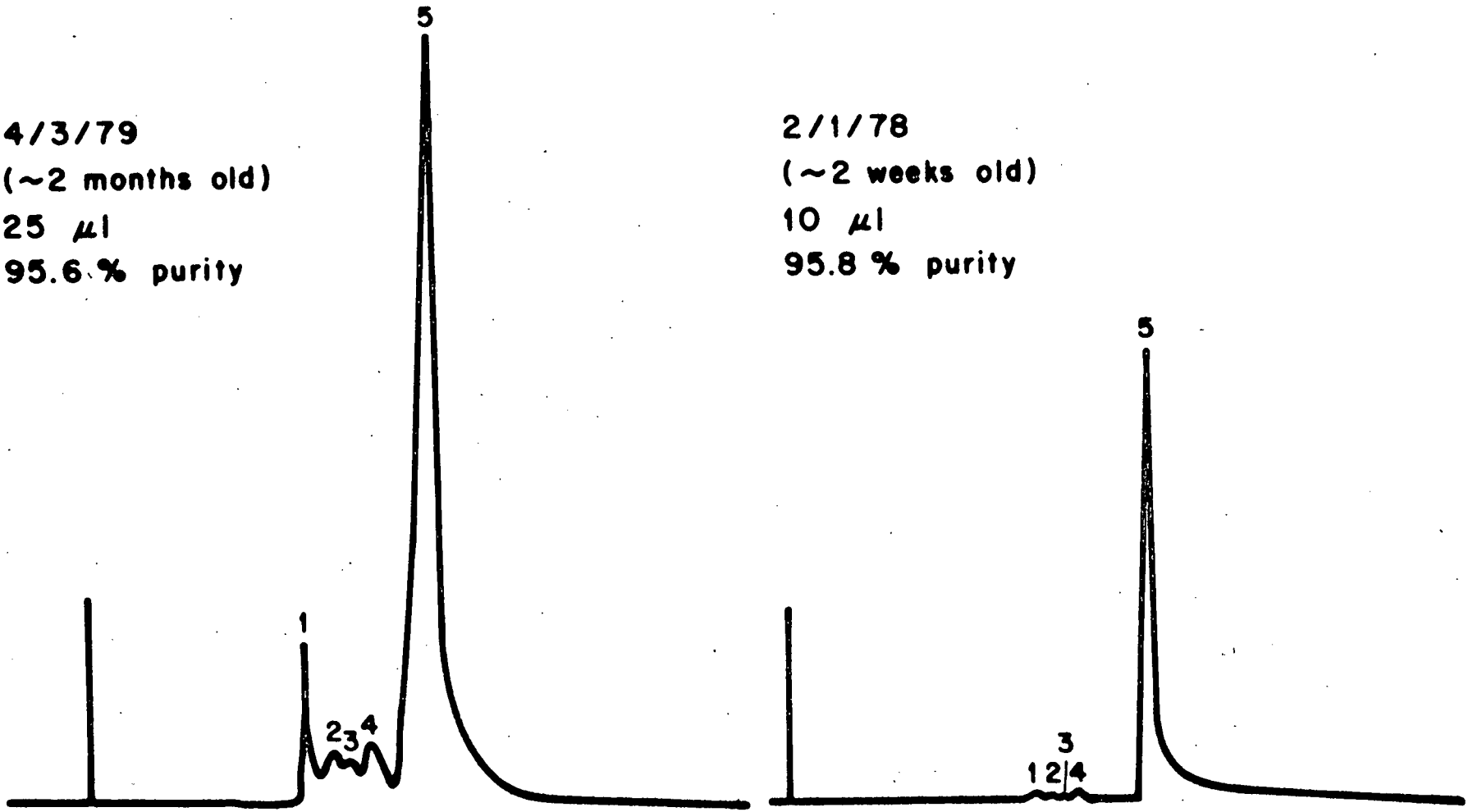



Figure 1.2.a-1
(See page 9-6)

Figure 1.2.a-1

The high pressure liquid chromatographic elution pattern of AA stock solution. Peak 5, (>96%) is pure AA and the peaks 1-4 are the impurities. 100% acetonitrile, at a rate of 2 ml/min. was used as a mobile phase.

aqueous system.

After the incubation period the excess of Hcl is neutralized by 0.1 M NaOH and the radioactive AA is taken up in 50% propylene glycol solution in 0.05 M phosphate buffer, pH 6.5, for injection. The small quantities of propylene glycol used are nontoxic and the final product may be injected into experimental animals without demonstrable reaction.

Quality Control

1. Radiochemical: This is checked by ascending paper chromatography using 99.9% methanol and 0.1% glacial acetic acid as a solvent. The unreacted $I^-$ moves to Rf value 0.6 and the iodinated AA migrates with the solvent front.

2. Biological: By biological control we mean determination of the possible alterations in the biochemical properties of radioiodinated AA.

We would anticipate that like unsaturated fatty acids (e.g. oleic acid) radioiodination of all the double bonds in AA would result in a loss of biological and metabolic properties. However, it is conceivable that the possible incomplete saturation described above may preserve some or all of the metabolic potential of AA.

It seemed to us that the initial step to see of the radioiodinated AA we made was biologically active was to compare its myocardial distribution in relation to blood flow to that of $H_3$-AA in both normal and ischemic zones. We recognize that similar distributions would not confirm preservation of metabolic activity, but clearly dissimilar distribution would indicate that a gross alteration of structure and biologic properties of AA had occurred because of radioiodination.

We describe our initial distribution studies below, and our planned in vivo and in vitro evaluation for metabolic function in the renewal proposal.

In Vivo Tissue and Myocardial Distribution Studies of Radioiodinated AA

1. Distribution of Radioactivity in Normal Canine Myocardium:

Three healthy, mongrel adult dogs were anesthetized with i.v. injection of pentobarbital (30 mg/kg body weight) and positioned under a Searle pho gamma HP camera equipped with a medium energy, high resolution collimator. Approximately 2 mCi of I-123 labeled AA were injected i.v. in a foreleg, left anterior oblique cardiac images were taken every 5 minutes for 30 minutes and then the animals were sacrificed with an excess dose of pentobarbital.

The hearts of the animals were quickly excised, washed free of blood, and sectioned into 1 cm thick transverse slices. These were imaged with a parallel hole collimator. A typical image shown in Figure 1.2.a-2 indicates the distribution of I-123 AA in myocardium. Approximately 8% of the administered radioactivity accumulates in normal canine, myocardium.

2. Distribution of Radioiodinated AA in Other Tissues and Clearance from Circulation

Tissue distribution studies of radioiodinated AA were performed in three healthy dogs. After i.v. injection of radioiodinated AA, serial venous blood samples were obtained and animals sacrificed at 2 hours post-injection. Various tissue samples were dissected and associated radioactivity counted together with a standard sample of radioactivity. The results were expressed as percent administered radioactivity per gram of tissue and per whole organ. Radioactivity in the thyroid of animals was also assessed. At 2 hours post injection, $<1\%$ of the radioactive iodine was found in the thyroid. Furthermore, a linear plot of blood clearance (Figure 1.2.a-3) shows that almost 90% of the administered radioactivity cleared from circulation within 10 minutes. At 2 hours post injection less than 1% of the radioactivity was circulating in the blood. We conclude that the rapid blood

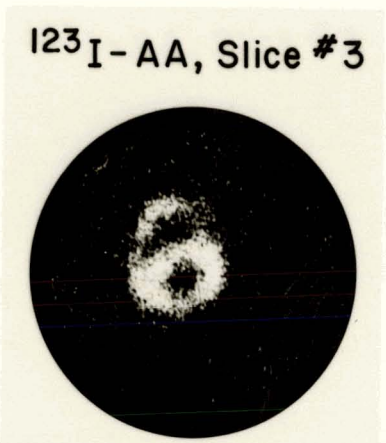



Figure 1.2.a-2

Gamma camera scintigraph of a 1 cm thick transverse slice of a normal canine heart obtained 30 minutes after an i.v. injection of 2 mCi I-123 AA. The circular area of activity at the bottom of the image represents the left ventricle, the thinner curve linear activity above is the right ventricle.

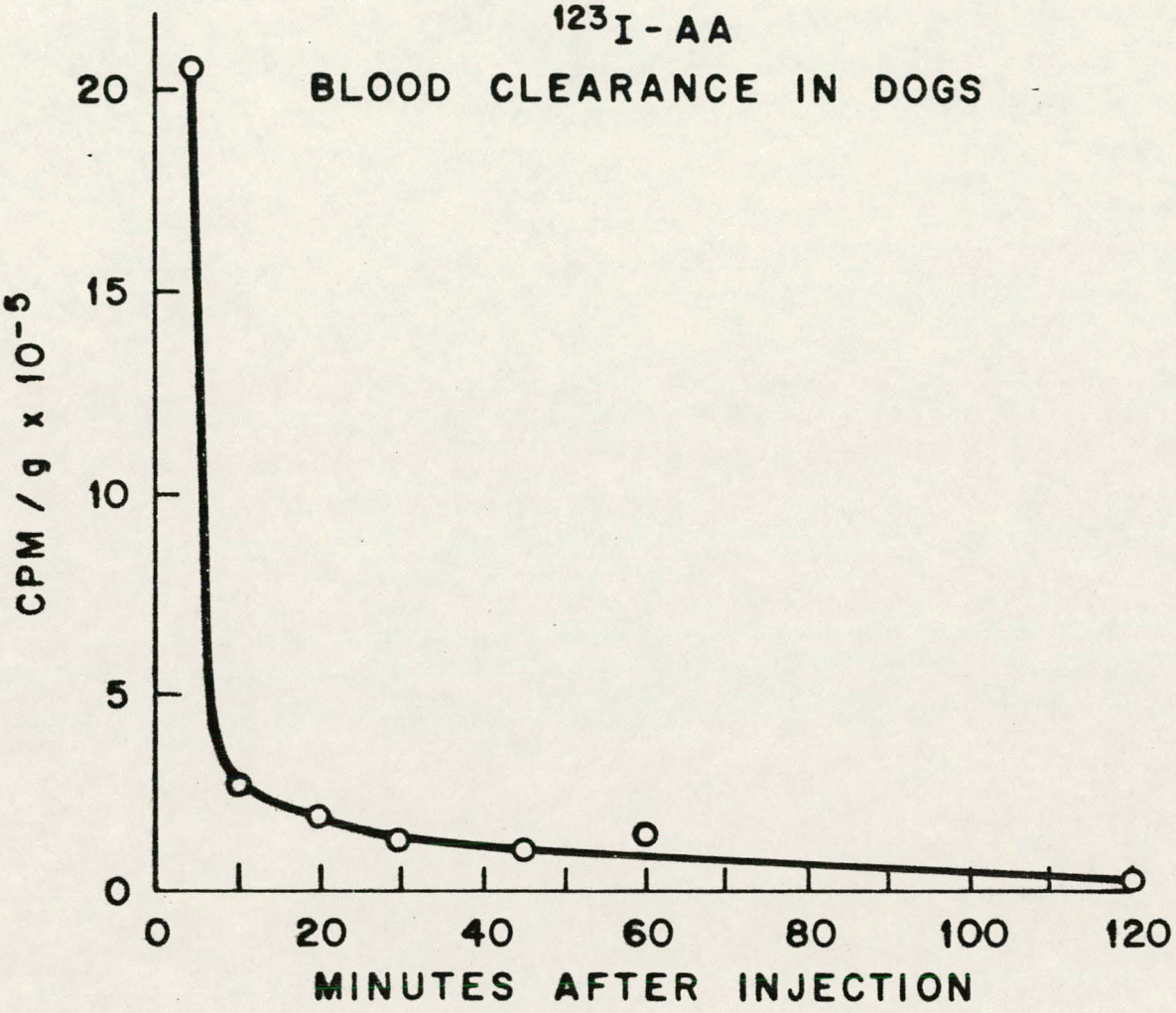



Figure 1.2.a-3

Blood clearance of radioiodinated AA after intravenous injections into the dogs.

clearance with insignificant thyroid uptake indicates that the AA is not deiodinated in vivo.

The tissue distribution of radioiodinated AA in dogs at 2 hours post injection is given in Table 1.

Table - 1

DISTRIBUTION OF RADIOIODINATED AA IN DOGS

| Organ | Two Hours Post Injection (% of administered dose) | |
|---|---|---|
| | % dose/g | % dose/total organ |
| Liver | 0.044 ± 0.001 | 46.95 |
| Spleen | 0.037 ± 0.005 | 6.48 |
| Kidneys | 0.044 ± 0.01 | 4.66 |
| Thyroid | 0.24 ± 0.03 | 0.56 |
| Myocardium | 0.029 ± 0.021 | 8.03 |
| Lungs | 0.021 ± 0.005 | 7.64 |

3. Uptake in Ischemic Myocardial Tissue and Distribution as a Function of Regional Myocardial Blood Flow:

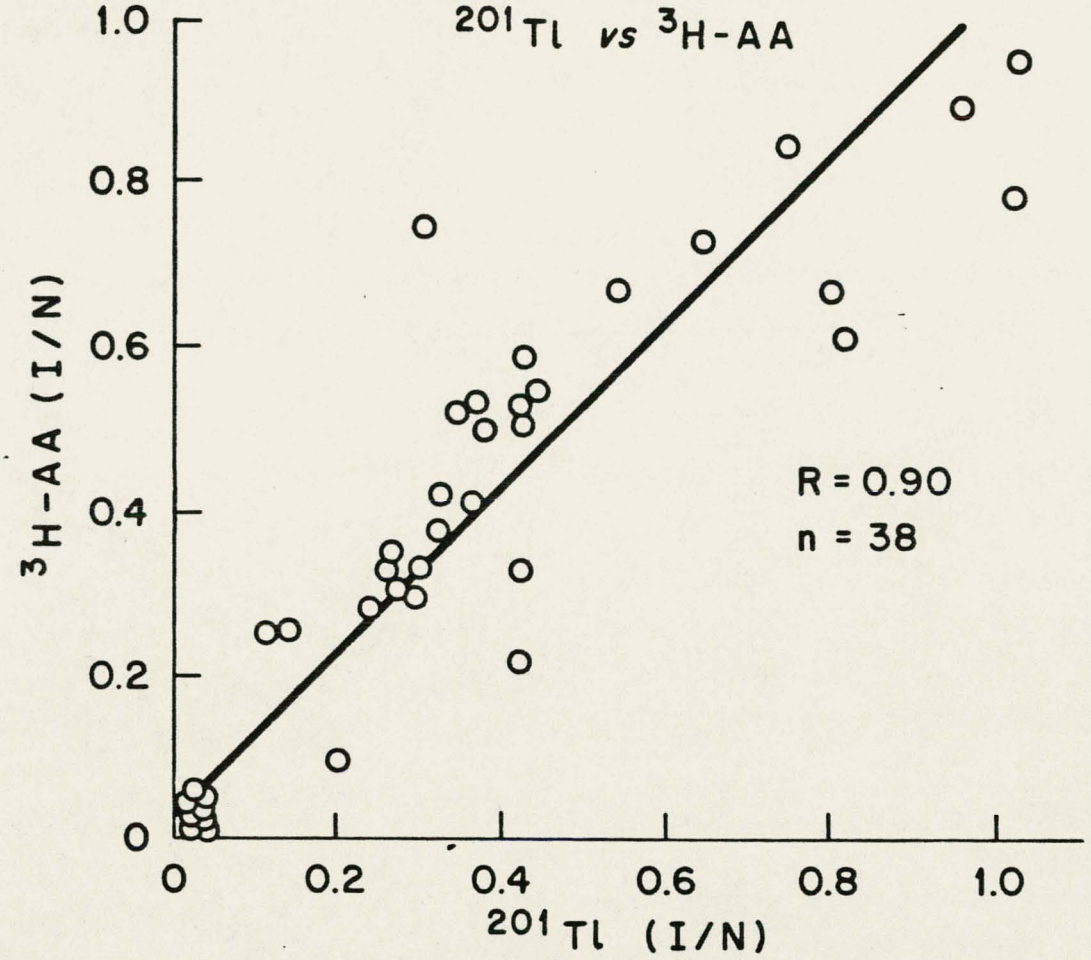
These experiments were designed to study the distribution of radioiodinated AA and compare it with the distribution of H-3 AA in ischemic canine myocardium as a function of regional myocardial blood flow. Two groups of three animals in each were studied.

Animals were anesthetized with 30 mg/kg sodium pentobarbital and anterior

wall myocardial infarction was induced by ligation of the left anterior descending coronary artery. Electrocardiograms were monitored continuously, and the animals were allowed to stabilize for 1 hour.

To the first group of three animals, approximately 1 mCi of I-123 labeled AA was administered i.v. Five minute later, approximately 400 μCi Sr-85 labeled carbonized microspheres were administered in the left atrium, allowed to equilibrate for two minutes, and the heart was exterpated. It was washed free of blood and kept cold in an ice bath. Approximately 20 samples were obtained from the grossly evident infarct zone. Six samples were also obtained from normal myocardium in the region of the circumflux coronary artery. The I-123 and Sr-85 radioactivity in each sample was then determined using a 3-channel automatic gamma counter. Suitable electronic windows were opened for 159 keV and 514 keV characteristic gamma photons of I-123 and Sr-85 respectively. Standard solutions of each radionuclide were also counted to determine the contribution of each radionuclide in other window. The samples were allowed to stand for 2 weeks to allow I-123 and contaminating I-124 (about 1% at the time of use, t ½ - 4.5 d, β+ (24%) to decay. The samples were counted again. Appropriate corrections were made and I-123 and Sr-85 radioactivity in each sample was computed. The accumulation of I-123 (representing AA distribution) and Sr-85 (representing relative regional myocardial blood flow) were then expressed as radioactivity uptake ratios obtained by comparing activity in samples from the abnormal zone to the mean activity from the six samples obtained from normal myocardium. The I-123 ratios were then plotted (y-axis) as a function of Sr-85 ratios (x-axis) on a linear scale. The plot is shown in Figure 1.2.b-4.

In the second group of three dogs with ischemic myocardial injury induced similarly, myocardium tissue distributions were carried out with H3-AA for comparison.



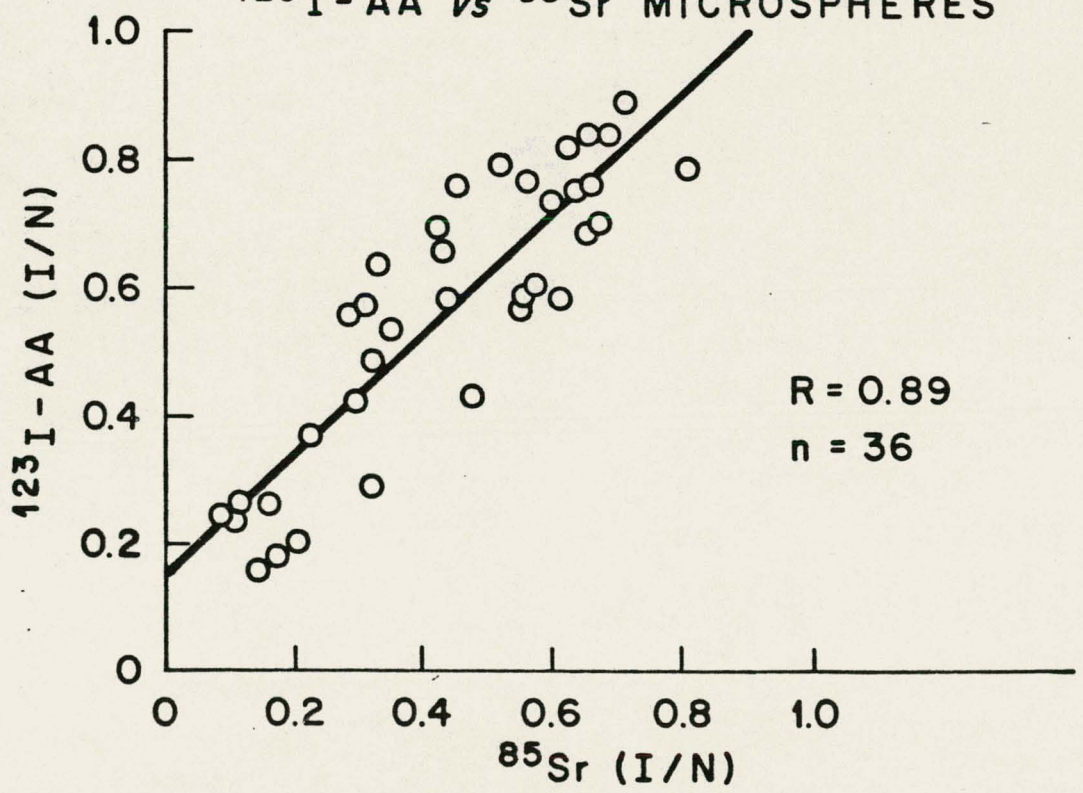




Figure 1.2.a-4

(See page 13.b)

Figure 1.2.a-4

Distribution of H-3-AA and I-123-AA studied in two separate groups of dogs subjected to a 60 minute acute coronary occlusion. Tl-201 in the H-3-AA group of animals Sr-85 microspheres in the I-123 AA group of animals were used as an indicator of regional myocardium blood flow. The ratios of H-3 radioactivity in abnormal (I) to normal (N) myocardium are plotted against the respective Tl-201 radioactivity ratios. A linear relationship between the two ratios is observed. (R=0.90, N=38). On a similar plot, the relationship between the (I/N) I-123 and (I/N) Sr-85 radioactivity ratios is also linear (R=0.89, N=36).

Thallium-201 was used as a blood flow indicator. Strontium-85 was not used since its Rb x-rays may contribute in H-3 counting and it has too long a half life (64 days) to allow it to decay. A protocol similar to that in the first group was used for obtaining myocardial samples and radioactivity calculations. A linear scale plot of H-3 radioactivity ratios (y-axis) versus Tl-201 radioactivity ratios (x-axis) is also shown in Figure 1.2.b-4.

The high degree of correlation between the two plots (0.9 and 0.89) suggests that the relation of radioiodinated AA distribution and myocardial blood flow is similar to that of H3-AA.

Thus, we have so far shown that:

1. AA can be radioiodinated with a high degree of labeling efficiency (95%).
2. Radioiodinated AA is not significantly deiodinated in vivo.
3. Up to 8% of the injected radioactivity is taken up by normal myocardium.
4. The radioiodinated AA clears rapidly from circulation.
5. Radioiodinated AA distribution in ischemic myocardium is directly proportional to blood flow and comparable to that of H3-AA ischemic myocardial distribution.

We believe these results indicate that no gross alteration of AA has occurred from radioiodination, and that we justified in proceeding on to evaluate the metabolic properties of radioiodinated AA. Our protocols for this will be described in the renewal proposal.

1.2b Simplified Method for Spleen Scanning with Tc-99m Labeled Erythrocytes

There are a number of conditions such as polysplenia, congenital asplenia, splenic trauma, splenic hypofunction and liver/spleen overlap for which selective spleen scanning is more efficacious than the routine 99m-Tc-colloid liver spleen scan. Selective spleen scanning is best done using 99m-Tc labeled denatured auto-

logous erythrocytes. With this problem in mind, Smith and Richards (J. Nucl. Med. 17:126 1976 ) have devised a kit for 99m-Tc-RBC's preparation. The kit, however, is not yet NDA approved, and requires washing the erythrocytes. We have modified their concept to develop a simple procedure that requires minimal manipulation of the blood, and can be performed with reagents available in any hospital radiopharmacy.

A commercially available bone seeking agent ("cold" pyrophosphate) is dissolved in sterile saline, injected into the patient, and approximately 30 minutes later, 6-8 ml of blood is drawn in a heparinized syringe. The blood is then transferred into a sterile Falcon, 15 ml test tube and 4-5 ml of pertechnetate-99m in 2-3 ml 0.9% NaCl is added to the blood. The mixture is then incubated for 35 minutes at $49^{o}C$, drawn back into a syringe and injected into the patient. Scintigraphy can be performed 1-2 hours later. Greater than 95% labeling efficiency is commonly achieved, and spelnic visualization studied in a small patient series has been excellent. Of note are patients evaluated for splenosis or a "born again" spleen who had had questionable splenic uptake on routine colloid liver/spleen studies but have obvious splenic localization with the above technique.

To eliminate the step of injecting "cold" bone seeking agent, we have attempted to add a calculated amount of the agent to the blood sample in vitro, before incubation with 99m-$TcO_4^-$. However, this results in much lower labeling efficiency (up to 80%), which we believe undesirable.

The work was carried out in collaboration with a second year nuclear medicine resident, Dr. R. Armas, and was presented in the 64th RSNA meeting (1978). A preprint of his article (Radiology 132:215-216 1979) is enclosed in Section 4.6 / of this report. The initial clinical results are presented to the 26th Annual Meeting of the SNM in June, 1979 (J. Nucl. Med. 20:683-684, 1979).

1.2c Radiolabeled Desferrioxamine (DF) Complexes. Evaluation of Relative Stabilities

Desferrioxamine type of siderophore, commonly known as bacterial growth factor, is produced by bacteria. Siderophores act as selective and strong chelating agents for $Fe^{3+}$ and produce 1:1 complex with a stability constant of $10^{30}$. As a result, Df has been used as a chemotherapeutic agent to reduce iron load in patients suffering from thalassemia. Gallium-67 citrate, a common clinical tracer in nuclear medicine, acts as an iron analogue when bound to transferrin. Gallium scintigraphy could be improved by reducing normal physiologic uptake in gut, liver, and skeleton. Our colleague, Dr. Paul B. Hoffer, interested in the potential use of DF clinically to reduce normal physiologic uptake of Ga-67 prior to scintigraphy, asked us to investigate relative stability constants of DF and transferrin complexes.

We therefore prepared In-111 and Ga-67 DF complexes and studied their relative stabilities with respect to Fe:DF, Cu:DF and EDTA and transferrin complexes of In-111 and Ga-67. Chromatographic and equilibrium dialysis techniques were employed.

Our data from these studies indicated that:

1. Ga-67:DF complex can be readily prepared over a wide pH range at room temperature.
2. Formation of In-111:DF is slow and dependent on pH and temperature.
3. The relative stability constants under our experimental conditions were GaDF > FeDF > InDF > CuDF.
4. At physiological pH, GaDF is a stronger complex than GaEDTA and is also stronger than Ga transferrin complex.

The work on the determination of the relative stabilities was presented to the 2nd International Symposium on Radiopharmaceuticals in March 1979 in Seattle by one of our colleagues, Dr. R. Weiner, and will be published as part of the proceedings of the meeting. A preprint of this article is enclosed in Section 4.7 of this report.

1.3 Interaction with Dr. Al Wolf and Colleagues in the Chemistry Department of the Brookhaven National Laboratories

The first project undertaken at the Brookhaven National Laboratory with Dr. Wolf was to develop an effective production technique for Rb-81 and to prepare an efficient generator for the elution of Kr-81m. This work was based on technology developed at Hammersmith Hospital (Jones et al., J. Nucl. Med. (1970)) where they made Rb-81-Kr-81m generators for clinical use in Europe (Fazio, Jones, Brit. Med. J. 265:673 (1975)).

The $^{79}Br$ ($\infty$,2n) $^{81}Rb$ reaction using bromoform, $CHBr_3$ as a target and the $^{82}Kr$ (p, 2n) $^{81}Rb$ reaction using natural krypton as a gas target were investigated. The latter reaction is more favorable and produces 11.6 $\pm$ 0.7 mCi Rb-81 uAhγ at the end of bombardment with 32 MeV incident proton beam energy. Various parameters, such as stationary phase, mobile phase and their flow rates, etc. for efficient elution of the 13 sec. t ½ Kr-81m gas were evaluated. Our final system for loading Rb-81 remotely on the column has now been fully automated. The report of this work has been accepted for publication in the Int. J. Appl. Radiat. Isotopes and a preprint has been enclosed in Section 4.8. This has enabled BNL to supply generators regularly to some medical centers on the East Coast.

Our interaction with BNL continues to grow. Since May of this year, we have already started a new project which will involve short lived positron emitting radiopharmaceuticals which will require not only the interaction of our radiochemical group with the BNL chemistry department, but also interaction of our nuclear cardiologists with the BNL medical group utilizing the PETT III for quantitative in vivo determinations in experimental animals. This is described in the renewal proposal.

2. Plans for the Continuation of Present Objects and Possible New Objectives in Consideration of Past Results

These are included separately in the renewal proposal.

3. Graduate Students Trained, Degrees Granted and Postdoctoral Tenures Completed

Two mecical students worked in our laboratory in conjunction with their undergraduate research theses for the Yale School of Medicine.

1. Dr. Marcia Wade - M.D. (1978)
   Principal Supervisor - Dr. John Dwyer (Immunology)

2. Dr. Craig Masson - M.D. (1979)
   Principal Supervisor - Dr. James Puklin (Ophthalmology)

The work described in 1.2b was carried out in collaboration with one of our residents, Renato Armas, M.D.,who completed a two-year nuclear medicine residency at Yale (1979), eligible for ABNM examination, September, 1979.

4. Bibliography of publications with title of publications associated with contract:

ABSTRACTS PUBLISHED

1. In vitro evaluation of Indium-111 labeled neutrophils: Mobility, chemotaxis and microbicidal activity. M.L. Thakur, B. Zakhireh, M. Cohen, A. Gottschalk and R. Root, Proceedings of the 25th Annual Meeting of the Society of Nuclear Medicine, J. Nucl. Med. 19, 672, 1978 (Abstract).

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3. Identification of Ga-67 birding component in human neutrophils. R.E. Weiner, P.B. Hoffer, and M.L. Thakur, Proceedings of the 25th Meeting of the Society of Nuclear Medicine, J. Nucl. Med, 19, 732 1978 (Abstract).

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13. Indium-111 desferrioxamine complexes: Preparation and stability studies. M.M. Goodman, M.L. Thakur, P.B. Hoffer, A.L. Riba, A. Gottschalk. J. Labelled Compounds 16:1, 1979 (Abstract).

14. Arachidonic acid. A potential myocardial imaging agent: Organ distribution and relation to regional myocardial perfusion. H.J. Boyer, M. Addabo, M.L. Thakur et al., Clin. Research 27:153A, 1979 (Abstract).

15. A simplified method of selective spleen scanning with Tc-99m erythrocytes: Clinical . R.R. Armas, M.L. Thakur, A. Gottschalk. J. Nucl. Med. 20, 1979 (Abstract).

16. Relative stability of In-111 and Ga-67 desferrioxamine (DEF) and transferrin (TF) complexes. R.E. Weiner, M.L. Thakur, M. Goodman and P.B. Hoffer, A.J.R. 132:489, 1979 (Abstract).

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1. Technetium-99m stannous pyrophosphate. Imaging of experimental endocarditis. A.L. Riba, J. Downs, M.L. Thakur et al., Circulation 58:111-119, 1978.

2. Imaging experimental infective endocarditis with In-111 labeled blood cellular components. A.L. Riba, M.L. Thakur, A. Gottschalk, et al., Circulation 59:336-343, 1979.

3. Ventilation patterns mimicking COPD patients with diaphgram pacing for Ondine's Curse. M.C. Makhija, H.J. Bronfman, R.C. Lange, W.L. Glenn, A. Gottschalk. Radiology 129:111-116, October, 1978.

4. Viability, random migration, chemotaxis, bactericidal capacity and ultrastructure of Indium-111 labeled human polymorphonuclear leukocytes. B. Zakhireh, M.L. Thakur, H.L. Malech, M.S. Cohen, A. Gottschalk, R.K. Root. Journal of Nuclear Medicine 20:741-747, 1979.

PAPERS IN PRESS

1. Imaging experimental myocardial infarction with In-111 labeled autologous leukocytes: Effect of infarct age and residual regional myocardial blood flow. M.L. Thakur, A. Gottschalk, B.L. Zaret. In press. Circulation.

2. Role of radiopharmaceuticals in nuclear hematology. M.L. Thakur, A. Gottschalk. Proceedings of 2nd International Symposium on Radiopharmaceuticals.

3. Relative stability of In-111 and Ga-67 desferrioxamine and human transferrin complexes. R.E. Weiner, M.L. Thakur, M. Goodman, P.B. Hoffer. Proceedings of 2nd International Symposium on Radiopharmaceuticals.

4. A simplified method for spleen scanning with 99m-Tc-labeled erythrocytes. R. Armas, M. Thakur, A. Gottschalk. In press. Radiology.

Cyclotron isotopes and radiopharmaceuticals: Aspects of production, elution, and automation of $^{81}Rb$ - $^{81}Krm$ generators.T.J. Ruth, R.M. Lambrecht, A.P. Wolf, M.L. Thakur. In press. International Journal of Applied Radiation and Isotopes.

Imaging experimental coronary artery thrombosis with Indium-111 platelets, A.L. Riba, M.L. Thakur, A. Gottschalk, B.L. Zaret. In press. Circulation.

The following reprints and preprints referred to in the text have been enclosed in appendix starting on page 25.

4.1 Viability, random migration, chemotaxis, bactericidal capacity and ultrastructure of Indium-111 labeled human polymorphonuclear leukocytes. B. Zakhireh, M.L. Thakur, H.L. Malech, M.S. Cohen, A. Gottschalk, R.K. Root. Journal of Nuclear Medicine 20:741-747, 1979.

4.2 Imaging experimental myocardial infarction with In-111 labeled autologous leukocytes: Effect of infarct age and residual regional myocardial blood flow. M.L. Thakur, A. Gottschalk, B.L. Zaret. In press. Circulation.

4.3 Technetium-99m stannous pyrophosphate. Imaging of experimental endocarditis. A.L. Riba, J. Downs, M.L. Thakur, et al. Circulation 58: 111-119, 1978.

4.4 Imaging experimental infective endocarditis with In-111 labeled blood cellular components. A.L. Riba, M.L. Thakur, A. Gottschalk, et al. Circulation 59:336-343, 1979,

4.5 Imaging experimental coronary artery thrombosis with Indium-111 platelets. A.L. Riba, M.L. Thakur, A. Gottschalk, B.L. Zaret. In press. Circulation.

4.6 A simplified method for spleen scanning with 99m-Tc-labeled erythrocytes. R. Armas, M. Thakur, A. Gottschalk. Radiology 132:215-216, 1979.

4.7 Relative stability of In-111 and Ga-67 desferrioxamine and human transferrin complexes. R.E. Weiner, M.L. Thakur, M. Goodman, P.B. Hoffer. Proceedings of 2nd International Symposium on Radiopharmaceuticals.

4.8 Cyclotron isotopes and radiopharmaceuticals: Aspects of production, elution, and automation of $^{81}Rb$-$^{81}Krm$ generators. T.J. Ruth, R.M. Lambrecht, A.P. Wolf, M.L. Thakur. In press. International Journal of Applied Radiation and Isotopes.

4.9 Role of radiopharmaceuticals in nculear hematology. M.L. Thakur, A. Gottschalk. Proceedings of 2nd International Symposium on Radiopharmaceuticals.

4.10 Ventilation patterns mimicking COPD patients with diaphgram pacing for Ondine's Curse. M.C. Makhija, H.J. Bronfman, R.C. Lange, W.L. Glenn, A. Gottschalk. Radiology 129:111-116, October, 1978.

5. Your Opinion as to the Present State of Knowledge in this Area of Research, Its Significance in the Fields of Biology and Medicine, and Needed Future Investigations

During the past 27 months (i.e. since Dr. Thakur came to Yale) the thrust of our work has dealt with the following:

Research Using In-111 Labeled Cellular Blood Components

We have established that under our experimental conditions, the radiolabeling of human polymorphonuclear leukocytes (PMN) with In-111 oxine does not cause any noticeable alteration in functional ability. These experimental data provide a foundation for using In-111 PMN in vivo applications.

Our own work studying the kinetics of PMN infiltrate in acute myocardial infarction was greatly facilitated by the use of In-111 tracer. This provided a method for quantifying PMN infiltrates in vivo as a function of infarct age and regional residual blood flow. We have already begun preliminary investigations in humans with MI using autologous In-111 PMN. The ultimate aims are to 1) characterize the inflammatory response of acute MI and 2) study the effect of therapeutic interventions designed to alter the inflammatory response. Furthermore, In-111 PMN's may be useful in the study of other cardiac inflammations such as pericarditis and myocarditis.

Scintigraphs of induced infective endocarditis in rabbits and coronary arterial thrombosis in dogs with In-111 labeled platelets represent extension of the potential scope of cardiovascular nuclear medicine. The results in animals are very encouraging and warrant clinical trials. For studies in man, however, the platelet labeling procedure needs to be modified. For all our animal work, platelets were washed and suspended in physiological saline prior to labeling with In-oxine. However, human platelets treated similarly rapidly clear from circulation (Thakur, unpublished data,

Goodwin, et al., J. Nucl. Med. 1978 ).

The use of plasma as suspending medium (Goodwin et al., 1978 and Scheffel et al., J. Nucl. Med. 20:524, 1979) yields lower labeling efficiencies. To overcome this problem, Welch et al. (private communication) use citrated normal saline (pH 6.5) as a labeling medium. Recently, however, it has been shown (Joist et al., J. Lab. Clin. Med. 92: (1978)) that glucose is essential to maintain platelet viability during labeling with In-111-oxine. In our laboratory we have begun investigations to develop a new method for labeling human platelets which will be mentioned in the renewal proposal.

New Radiopharmaceuticals

Our initial joint efforts at the Brookhaven National Laboratory have resulted in making Rb-81-Kr-81m generators available regularly to a few centers on the East Coast. In addition, we have systematically studied parameters that enabled us to provide an optimal generator.

Our modified heat treated red cell labeling procedure for spleen imaging can be performed easily in any nuclear medicine laboratory. The procedure is simple and does not involve any agent that is not currently available commercially.

Much effort during the past few months have been made in developing arachidonic acid (AA) to study cardiac conditions. So far, we have shown that AA can be radioiodinated and its in vivo and cardiac distribution is similar to that of H-3 AA.

A series of in vitro tests are planned to ascertain whether radioiodinated AA will maintain its metabolic properties and act as a precursor of prostaglandins.

We will need the BNL facilities to complete our investigation of radioactive AA as a potential tracer for cardiac pathophysiology and metabolism, and have detailed these plans in the renewal proposal.

6. The Present Division of Federal Support for Your Overall Research Program

Our overall research program is carried out in collaboration with Dr. B.L. Zaret, Chief of Cardiology Section in this institution. His research program is supported in part by the RO/HL 21690-02 from the National Heart, Lung and Blood Institute, with an annual (1978-1979) award of $121,478. Dr. A. Gottschalk is a Co-Investigator of this research program, but derives no salary support.

We have also collaborated (Section 1.2.c. of this report) with Dr. P.B. Hoffer, Chief of Nuclear Medicine in our department. His research is supported by DOE Contract, EP-78-S-02-4625. The annual (1978-1979) budget of this award is $99,905. Neither Dr. Gottschalk nor Dr. Thakur derive salary support from this grant.

Dr. Gottschalk is Co-Principal Investigator (10% salary support) of a contract from the National Heart, Lung and Blood Institute, No. N01-HR-9-2904, with an award of $127,351 (1979-1980). Dr. R.H. Greenspan, Chairman of our department is the Principal Investigator.

APPENDIX 1

PUBLICATIONS

Reprints + Preprints Removed