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July 1984

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Prepared for the U.S. Department of Energy under Contract DE-AC03-76SF00098

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LBL Report #18388

Gallium-68 Chemistry for Labeling Platelets, Proteins and Lipoproteins

Y. Yano, T. F. Budinger, S. N. Ebbe, C. A. Mathis, D. H. Moore, M. Singh, K. Brennan, B. R. Moyer, and A. Nichols

Presented in part at the 5th International Symposium of Radiopharmaceutical Chemistry, Tokyo, Japan, July 9-13, 1984.

ABSTRACT

Generator produced gallium-68 is a convenient useful radionuclide for positron emission tomography (PET) investigations. Gallium-68 labeled platelets and low density lipoproteins would be useful agents for PET studies of thrombosis and atherosclerosis in cardiovascular disease. To label these agents with Ga-68, we have studied the effects of trace metal contaminants in 1 N HCl elutions of Ga-68 from germanium-68 adsorbed on a stannic oxide column. Studies were conducted on the formation and characteristics of Ga-68 complexes with the ligands 8-hydroxyquinoline, tropolone, and mercaptopyridine-N-oxide (MPO). Various parameters such as pH, buffers, concentration of ligand, and incubation or stability with time were investigated. High performance liquid chromatography and instant thin layer chromatography were used to analyze the Ga-68 ligand preparations.

Platelets separated from human, dog, and rabbit plasma were incubated with the Ga-68 complexes and the labeling yields and in vivo survival were determined. The accumulation of the platelets in the ballon catheter scraped aorta of the rabbit was determined by PET imaging studies, tissue counting in a gamma well counter, and en-face autoradiography of the arterial wall.

The Ga-68 complexes of MPO gave 40-60% labeling efficiency of rabbit platelets which accumulated about fourfold more in the damaged aorta compared to the normal.

Gallium-68 was attached to low density lipoproteins (LDL) with the bifunctional chelate of DTPA. Low pressure gel column chromatograpy and HPLC were used to preparatively separate and analyze the Ga-68 LDL for uptake studies in the healing endothelium of the scraped aorta rabbit model. The Ga-68 LDL labeling yield was 80-85% with a radiochemical purity 90-95%.

INTRODUCTION

The 68 min positron emitter Ga-68 is readily obtained as ionic Ga+3 by elution with 1 N HCL from the 275 day half-life germanium-68 parent isotope adsorbed onto a stannic oxide column (1,2). Because trace metals in the Ga-68 eluate compete with the carrier-free Ga-68 for the complexing ligand, organic solvent extraction and anion exchange resin separations are necessary to decrease the concentration of trace metals (3,4).

Since Ga+3 is readily hydrolyzed even in pH 3-4 aqueous solution, a weakly complexing acetate or citrate buffer is added to the Ga-68 in 0.01 N HCL solution prior to ligand addition and pH adjustment to 6-7 to prevent the formation of $Ga(OH)_3$.

Thakur and Welch used 8-hydroxyquinoline (oxine) for labeling blood cells and platelets with Ga-68 and In-111 (5). Subsequently Thakur investigated the use of mercaptopyridine-N-oxide as the complexing ligand for In-111 (6). Dewanjee used tropolone to label platelets, neutrophils, and leukocytes with In-111 which has chemical properties similar to Ga-68 (7).

In this study the formation of the Ga-68 complex was determined by ITLC and HPLC and added to separated platelets. After incubation the Ga-68 labelled platelets were separated by centrifugation, washed with saline, and injected into the rabbit model. The uptake of the Ga-68 platelets in the normal and freshly scraped aorta was determined by PET imaging and by direct counting of the blood vessels in a NaI(Tl) well counter.

The cyclic anhydride of DTPA was conjugated to low density lipoprotein (LDL) by the methods of Eckelman and Hnatowich (8,9). Ga-68 was chelated by the DTPA moiety, which was itself covalently bound to an amino group of the LDL protein. The LDL was obtained from human plasma by the ultracentrifugation method of Nichols et al. (10). The LDL-DTPA conjugate and the Ga-68 DTPA-LDL were separated by gel column chromatography and HPLC (11). Ga-68 LDL was injected into the scraped aorta rabbit model to measure the accumulation of the labeled LDL in the healing endothelium 20-30days post injury. The scraped aorta provides a convenient model of atherosclerotic lesions (12).

Endothelial injury and thrombosis are involved in the development of both the early and advanced lesions of atherosclerosis and in thromboembolic complications of atherosclerosis (13). Endothelial injury may be brought about by blood-borne factors and by effects of blood flow which affects the vessel wall and the endothelium. Blood-borne factors are serum lipids (particularly LDL), high concentrations of abnormal metabolites such as homocysteine,

antigen-antibody complexes, catecholamines, bacteria and viruses. It is well known that platelet deposition occurs at the injury sites on the arterial wall and that LDL is taken up by specific cells via a receptor mediated process (14). Thus, the radiolabeling of platelets and LDL with Ga-68 may permit the imaging of their accumulation with high resolution positron emission tomography for the evaluation of vascular lesions (15).

MATERIALS AND METHODS

A 25 mCi Ge-68/Ga-68 generator (New England Nuclear) was used over a period of nearly two years for these studies. The cummulative elution yield of Ga-68 was 2.3 Ci for 250 separate elutions with a total volume of 1500 ml of 1 N HCl eluent. The elution yield of Ga-68 per 2.5 ml of eluent was 75-85% of the maximum and the breakthrough of Ge-68 was 10^{-4}%. The breakthrough of the Sn adsorber was <1 ppm. Reagent grade chemicals (Sigma, Aldrich) were used for these studies. In addition the hydrocloric acid was purified by an anion exchange resin column (Bio Rad AG 1X8, 100-200 mesh). All glassware used in these experiments was acid washed and rinsed thoroughly with deionized water. Other trace metals in the eluate were reduced in concencentration by ether extraction from a 6 N HCl solution of $GaCl_4^-$ using a MIXXOR™ liquid-extractor. The ether phase was collected and evaporated to dryness under a filtered nitrogen stream and in a warm water bath. The Ga-68 was reconstituted in 0.5 ml of 0.01 N HCl and 300-500 µl of 0.5 M sodium acetate was added to raise the pH to about 5.0. The complexing ligands used in these studies were 8-hydroxquinoline (oxine), tropolone (Tro), and mercaptopyridine-N-oxide (MPO) Fig. 1. The oxine was prepared in ethanol at a concentration of 1 mg/ml. The tropolone and sodium-salt of MPO were prepared in water at the same concentration as oxine. Twenty to four hundred µl of the ligand were added to the Ga-68 acetate solution and the pH adjusted to 6.5-7.5 with dilute NaOH.

Analysis: In this study the formation of Ga-68 complexes was quantitatively determined by ITLC for oxine and tropolone. Gelman SG ITLC strips were used as the support medium and $MeOH:CHCl_3$ (5:95) was the developing solvent. The Ga-68 MPO was analyzed by HPLC using a C-18 reverse phase column and $CH_3OH:H_2O$ (25:75) in 10 mM citrate at pH 3.5 as the solvent at a flow rate of 1-2 ml/min. Both 254 nm UV and gamma radioactivity detectors were used with an integrating chart recorder to quantify the Ga-68 ligand formation.

Platelet Preparation: The platelets used in these studies were obtained from 35-45ml of human, dog, or rabbit blood that was collected in Squibb modified acid citrate dextrose (ACD) solution in a plastic tube (later modified to low

volume plastic bag). The capped tube was centrifuged at 170-200g for 15 min and the platelet rich plasma (PRP) was transferred with a plastic pipet into a plastic tube and centrifuged at 700-1100g for 15 min. The platelet poor plasma was removed and the platelet button was resuspended in 2.0-.3.0 ml of normal saline at pH 6.5-7.5, recentrifuged and resuspended in saline. The Ga-68 MPO was added to the platelet suspension and incubated at room temperature for 15 min. The Ga-68 platelets were again centrifuged for 15 min, the supernatant removed, and the radioactivity in the platelet button and the supernatant was measured by a Capintec dose calibrator. A second resuspension in saline was followed by a centrifugation and separation of the platelet button to measure the amount of labile Ga-68. The platelets were resuspended in 4-5ml of normal saline for administration to the animal model.

Low Density Liproprotein Preparation: The preparation of the labeled LDL is shown in the flow diagram in Fig. 2. Human LDL was isolated from a freshly drawn unit of blood containing 0.5% EDTA as an anticoagulant. The plasma fraction was separated by centrifugation (1200g at $4^{o}C$) and penicillin streptomycin and ethylmercurisalicylic acid were added, and the LDL was separated from the plasma by ultracentrifugation (40,000g Beckman 40.3 rotor). The isolated LDL was stored under nitrogen at $5^{o}C$.

The cyclic anhydride of DTPA was prepared by the method of Hnatowich (19). An excess of acetic anhydride in pyridine was added to DTPA and heated at $65^{o}C$. The precipitate was collected, washed, dried, and analyzed by NMR spectroscopy and elemental analysis. The results for elemental analysis were: C calc. 46.1, found 46.1, found 46.8, H calc. 5.32, found 5.21, N calc. 11.9, found 11.8 and O calc. 35.8 found 36.2. The melting point (178-$183^{o}C$) is within the published range. The coupling of the cyclic anhydride of DTPA to LDL was carried out by adding 0.1 mg of DTPA anhydride in 1 ml of dry $CHCl_3$ to 0.5 ml of LDL solution (10-14 mg/ml) and adjusting the pH to 7.0 with 0.5 M phosphate buffer.

The conjugated DTPA-LDL was purified by passage through a Sephadex G-50 gel column using 0.01 M Na_2HPO_4, 0.9% NaCl buffer at pH 7.4 as the eluent. Fractions of 0.5 ml volume were collected and assayed by U.V. (214 nm). The fraction with the highest concentration of the conjugated DTPA-LDL was used for labeling with Ga-68 acetate. The radiochemical purity of the LDL-Ga-68 was determined by HPLC using a BioGel TSK 50 column (7.5x300mm) and 0.01M Na_2HPO_4,0.9% NaCl at pH 7.4 as the eluent.

Animal Model Studies: New Zealand white rabbits weighing 3-4 kg were used in these studies. The scraped aorta rabbit models were prepared about 10-20

min before intravenous injection of the Ga-68 labeled platelets. An embolectomy catheter was introduced intrafemorally and pushed cranially to the aortic arch. The bulb was inflated and the aorta was scraped 3 times before the catheter was removed.

In later studies the Ga-68 labeled platelets were injected prior to scraping the aorta to minimize the uptake of non-radioactivity platelets on the exposed media layer.

The Ga-68 platelets were administered intravenously and whole body PET images were obtained with the 280 BGO detector positron tomograph (16). A 10 mm detector half gap was used with a 30 sec data accumulation time at each 10 mm bed position. A total imaging time of 20-30 min was required for each whole body scan. After imaging the aorta and vena cava in the cross-sectional mode, the animals were sacrificed and the per cent uptake of the injected tracer was determined for selected tissues as well as for the normal and scraped aorta by counting the various samples in a NaI(Tl) well counter. One rabbit had enface autoradiography of the aorta wall demonstrating the regional uptake of the labeled platelets on the exposed media smooth muscles layer. Normally intimal endothelium protects the medial layer from platelet adherence and degranulation. The removal of the endothelial layer from the vessel wall was confirmed by injection of Evans blue dye thirty minutes prior to the sacrifice (17).

Similar studies were done with rabbits and Ga-68 DTPA-LDL with the exception of waiting 20-30 days after scraping the aorta before injection of the Ga-68 LDL. This was to ensure that the endothelium was in the healing phase which is known to have the highest affinity for radioiodinated LDL (12). Both PET imaging and tissue counting studies were done in these studies at 2-3hr after i.v. administration of the tracer compound.

RESULTS AND DISCUSSION

The presence and removal of trace metals in the Ga-68 eluates from the SnO_2 column before and after ether extraction are shown in Table I. There was nearly a tenfold reduction in the concentration of some metals such as Al, Ca, Cu, Fe, Mg, and Zn.

The removal of trace metals by an anion exchange resin (Bio Rad AG1 X 8, 100-200 mesh) column clean-up of reagent grade hydrochloric acid is shown in Table II. There was a 5-10 fold reduction in Ca, Cu, Fe, and Zn. These results indicate that both anion exchange resin and ether extraction are effective methods of minimizing the competition for the ligand by trace metal contaminants. In an experiment similar to that of McElvany, et al. (3), we compared the formation of Ga-68 EDTA for the direct column eluate and the ether extracted Ga-68 for various concentrations of EDTA, Fig. 3. For concentrations of EDTA < 0.30 mg/2.5ml there was < 10% Ga-68 EDTA formation for the direct column eluate while for the ether extracted Ga-68 there was > 98% formation of Ga-68 EDTA for 0.08 mg EDTA/2.5ml. Clearly the removal of trace metal contaminants enhanced the formation of the Ga-68 chelate.

The known binding constants for In-111 and its congener Ga-68 with oxine are given in Fig. 1. It can be seen that for oxine the binding affinity is less by an order of magnitude for In-111 as compared to Ga-68. This difference might account for the higher labeling yields of platelets with In-111 as compared to Ga-68. It has been shown with H-3 labeled oxine and In-111 labeled platelets and neutrophils that the In-111 oxine complex diffuses freely across the cell membrane and into the cytoplasm. The complex dissociates, In-111 binds to a cytoplasmic protein and is retained within the cell while the oxine diffuses back across the cell membrane (18,19). In the case of Ga-68 oxine, the more strongly bound Ga-68 oxine complex can diffuse out of the cell prior to dissociation, and Ga-68 will not be trapped within the cell cytoplasm to the same extent as In-111.

Initial evaluation of Ga-68 oxine formation was by extraction into dichloromethane from a pH 6.5 aqueous solution of Ga-68 oxine prepared from 1 N HCl eluates. The per cent extraction of Ga-68 oxine as a function of oxine concentration and volume of Ga-68 generator eluate is shown in Fig. 4. With 50 μl (50 μg) of oxine there was about 96% extraction of Ga-68 oxine when 0.3 ml of 1 N HCl eluate was used; however the extraction decreases to about 75% when 0.7 ml of eluate is used. Conversely > 95% extraction of Ga-68 oxine is obtained when 100 μg or more of oxine is used with up to 1.0 ml of eluate. These results indicate that at low concentration of oxine there is competition

for the ligand by trace metals from increasing volumes of Ga-68 generator eluate.

In subsequent experiments, an ITLC method was used to determine the formation of Ga-68 oxine and tropolone as shown in Fig. 5. Also shown is the formation of Ga-68 MPO as determined by HPLC. These chromatograms were quantified by an integrating strip chart recorder connected to the output from the radioactive detector (RAD) from the HPLC or the RAD from the ITLC chromatogram scanner. Using the ITLC method for analysis, the stability of Ga-68 oxine to dilution volume and time after preparation (shelf life) are shown in Fig. 6. A standard preparation with 100 μg oxine and 0.5 ml of Ga-68 eluate was used to study the effects of time and dilution. The Ga-68 oxine was relatively stable for up to 30 min after preparation and stable to dilution up to 5 ml of volume.

Further studies were done to determine the optimum concentrations of Tro and MPO that gave > 90% formation of the Ga-68 complex. The flow diagram for the procedure and the HPLC analysis of Ga-68 MPO are seen in Fig. 7. The results of the Ga-68 platelet labeling studies are shown in Table III. In all cases the formation of the Ga-68 ligand complex was determined to be > 90% before attempting to label the platelets. Ga-68 oxine gave variable results in platelet labeling using human, dog, and rabbit platelets (1×10^9 - 1×10^{10}) suspended in ACD - saline, saline alone or platelet poor plasma. About 26-84% of the Ga-68 was labeled to the platelets with about 50% of the Ga-68 label being washed from the platelets in the first saline wash. There was 13-42% of the initial Ga-68 activity labeled to the platelets at the time of administration to the rabbit.

Ga-68 Tro did not label the dog platelets sufficiently to allow in vivo studies.

With Ga-68 MPO there was a more reproducible labeling yield of 43-63% with both human and rabbit platelets. Also, the first saline wash of the labeled platelets resulted in only a 10-20% loss of radioactivity indicating a more firmly bound (intra-cellular) label than with Ga-68 oxine. The platelets labeled with Ga-68 MPO were injected intravenously into normal and scraped aorta rabbit models to give the PET images in Fig. 8. The location of the aorta and vena cava was established by dynamic positron imaging at 2.5 sec intervals which showed initial filling of the aorta and then the filling of the vena cava. There was greater accumulation of Ga-68 platelets in the damaged descending aorta compared to the normal. In the cross-sectional view the Ga-68 platelet activity can be seen in the aorta and vena cava. Sequential imaging

of 5 min each were obtained up to 2 hours after injection of the Ga-68 platelets. The percent uptake of the Ga-68 platelets in selected organs and aorta is seen in Table IV. The spleen has the greatest concentration of the Ga-68 platelets. There was nearly four times (3.7) more uptake in the damaged endothelium of the aorta compared to the normal. The damaged aorta had about 4 times the uptake of Evans blue dye and radio-activity as determined by autoradiography and densitometer measurements Fig. 9.

Further studies are underway with Ga-67 labeled platelets to determine the biological viability of the labeled platelets by survival times (labeled platelet recovery) and aggregation to ADP or collagen stimulation.

The results of the Ga-68 bifunctional chelate labeling of LDL are seen in Fig. 10 which shows the HPLC analysis of the Ga-68 DTPA-LDL. Studies are also being conducted with LDL and Ga-67. The 3.2 day half-life of Ga-67 will permit analysis by electrophoresis and cell binding studies to fibroblasts to determine the physico-chemical state and biological viability of LDL to labeling with Ga-68 by the bifunctional chelate method. In addition radioiodinated LDL will be used as a control for the stability of LDL to radiolabeling with metal radionuclides.

These early studies indicate some promise for positron emitter labeled platelets and LDL. A new high resolution (2-3mm) PET system now under development may be helpful in studying discrete cardiovascular lesions with these imaging agents. Dual radioisotope studies with I-125 and Ga-68 LDL will be useful in determining LDL accumulation on intact and de-endothelialized aorta in rabbits perfused in situ (20).

It may be necessary to subtract a blood activity to visualize and quantify the platelet accumulation in the atherosclerotic lesions (21). If this is the case, a convenient blood volume agent would be I-122 labeled serum albumin. The I-122 isotope is generator produced and the ^{122}I albumin would be used to determine the blood volume in the aorta and to substract the blood platelet radioactivity so that the net platelet activity accumulating in the endothelium of the aorta could be determined (22).

Acknowledgements

TM - MIXXOR, The Lidex Corp. Jerusalem, Israel.

We thank G.V. Shalimoff of Lawrence Berkeley Laboratory for the trace metals analysis.

This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Biology and Medicine Division of the U.S. Department

of Energy under Contract No. DE-AC03-76SF00098 and also by the National Institute of Health, National Heart, Lung, and Blood Institute under Grant No. P01 HL25840-02.

Figure Legend:

1. Ligands for Ga-68 complex formation.
2. Preparation and labeling of DTPA-coupled human low density lipoprotein.
3. Ga-68 EDTA formation. Ga-68 from either direct SnO_2 column eluate or ether extracted.
4. Ga-68 oxine extraction into CH_2Cl_2 as a function of oxine concentration and Ga-68 eluate volume.
5. ITLC and HPLC analysis of Ga-68 oxine, Ga-68 tropolone, and Ga-68 mercaptopyridine-N-oxide formation. The Ga-68 MPO HPLC analysis was at flow rate of 2 ml/min. The retention volume is 3.38 ml for ionic Ga and 7.68 ml for Ga-MPO.
6. Ga-68 oxine stability, effect of dilution volume and time after preparation.
7. Fig. 8 (top) Flow diagram of Ga-68 MPO formation, platelet separation and Ga-68 platelet labeling. Fig. 8 (bottom) HPLC analysis of Ga-68 MPO with UV and radioactivity detector. The radioactive peak (solid line) retention time is slightly delayed from UV peak (broken line) because of the tubing volume from UV to radioactive detector; with this correction the retention time for UV and radioactivity coincide.
8. Ga-68 platelet imaging in scraped aorta rabbit model with a whole body positron emission tomograph.
9. Fig. 9. scraped and normal aorta showing Evans Blue staining and radioactivity by autoradiography and densitometer measurements.
10. HPLC analysis of Ga-68 LDL-DTPA by gel permeation chromatography with UV and radioactive detector. The delay in the radioactive peak from the UV peak is caused by the extra tubing volume from UV to NaI (Tl) detector.

Tables

I. Trace metals in direct column eluate and ether extracted Ga-68 from a SnO_2 Ge-68 generator.

II. Trace metals in reagent grade and anion exchange resin column purified HCl eluent and eluate of Ga-68.

III. Gallium-68 platelet labeling studies.

IV. Uptake of Ga-68 labeled platelets in scraped aorta and normal rabbits.

References

1. Loch C, Maziere B, Comar D. A new generator for ionic gallium. J Nucl Med 21:171-173, 1980.
2. Hanrahan TJ, Yano Y, Welch MJ, McElvany KD, Moore HA: Long term study of high level Ge-68 generators. Abstract, Fourth International Symposium on Radiopharmaceutical Chemistry, Julich, GFR, August 23-27, 1982, pp. 256-266.
3. McElvany KD, Hopkins KT, Welch MJ: Comparison of $^{68}Ge/^{68}Ga$ generator systems for radiopharmaceutical production. Int J Appl Radiat Isot 35: 521-524, 1984.
4. Goodwin DA, Bushberg JT, Doherty PW, Lipton MJ, Conley FK, Diamanti CI, and Meares CF: Indium-111 labeled autologous platelets for location of vascular thrombi in humans. J Nucl Med 19:626-634, 1978.
5. Thakur ML, Welch MJ, Joist JH, Coleman RE: Indium-111 labeled platelets: Studies on preparation and evaluation of in vitro and in vivo functions. Throm Res 9:345-357, 1976.
6. Thakur ML, Barry MJ: Preparation and evaluation of a new indium-111 agent for efficient labeling of human platelets in plasma. Fourth Intl. Symp. Radiopharmaceutical Chemistry, Julich, GRF, August 23-27, 1982, p. 140-141.
7. Dewanjee MK, Rao SA, Didesheim PJ: Indium-111 tropolone, a new high affinity platelet label: preparation and evaluation of labeling parameters. J Nucl Med 22:981-987, 1981.
8. Eckelman WC, Karesh SM, Reba RC: New compounds-fatty acid and long chain hydrocarbon derivatives containing a strong chelating agent. J Pharm Sci 64:704-706, 1975.
9. Hnatowich DJ, Layne WW, Childs RL: The preparation and labeling of DTPA-coupled albumin. Intl J Appl Radiat and Iso 33:327-332, 1982.
10. Nichols AV, Gong EL, Blanche PJ, Forte TM, Shore VG: Interaction of Model discoidal complexes of phosphotidyl choline and apolipoprotein A-1 with Plasma components. Biochim Biophys Acta 793:325-337, 1984.
11. Ohno Y, Okazaki M, Hara I: Fractionation of Human serum lipoproteins by high performance liquid chromatography. J Biochem 89:1675-1680, 1981.
12. Roberts AB, Lees AM, Lees RS, Strauss HW, Fallon JT, Taveras J, Kopiwoda S: Selective accumulation of low density lipoproteins in damaged arterial wall. J Lipid Research 24:1160-1167, 1983.
13. Mustard JF, Kinlough-Rathbone RL, Packham MA: In Clinical Diagnosis of Atherosclerosis, Eds. MG Bond, W Insull, Jr, S Glagov, AB Chandler, JF

Cornhill, Springer-Verlag, New York, 1983, Chapt. 22.

14. Brown MS, Kovanen PT, Goldstein JL: Regulation of Plasma Cholesterol by lipoprotein receptors. Science 212:628-635, 1981.
15. Budinger TF, Ganz E, Price DC, Lipton M, Moyer BR, Yano Y,: In Clinical Diagnosis of Atherosclerosis, Eds. MG Bond, W Insull, Jr, S Glagov, AB Chandler, JF Cornhill, Springer-Verlag, New York, 1983, Chapt. 10.
16. Derenzo SE, Budinger TF, Cahoon J, etal: High resolution computed tomography of positron emitters. IEEE Nucl Sci NS-24 (1) 544-558, 1977.
17. Alavi M, Moore S,: Kinetics of low density lipoprotein interactions with rabbit aortic wall following ballon catheter de-endothelializatiion. Atherosclerosis 4:395-402, 1984.
18. Thakur ML, Segal AN, Louis L, etal: Indium-111 labeled cellular blood components. Mechanism of labeling and intracellular location in human nuetrophilis. J Nucl Med 18:1022, 1977.
19. Mathias CJ and Welch MJ: Labeling mechanism and location of In-111 in human platelets. J Nucl Med 20:659, 1979.
20. Preobrazhensky SN, Dolgov VV, Flegel HG, Reprin VS, Smirnov VN,: [^{125}I] LDL uptake in rabbit arteries perfused in situ: Effect of HDL on intact and de-endothelialized vessels. Atherosclerosis 48:147-155, 1983.
21. Iaha Y, Kimura K, Yoneda S, Kusnoki M, Etani H, Uyama O, Tsuda Y, Abe H,: Platelet accumulation in carotid atherosclerotic lesions: Semiquantitative analysis with indium-111 platelets and technetium-99m human serum albumin. J Nucl Med 25:556-563, 1984.
22. Mathis CA, et al: in press.

Table I

Trace Metals, μg/100 μl, In ^{68}Ga Elutions, 1.0N HCl, SnO_2 Column

Element	Sample I[a]	Sample I[b]
Al	0.1	<0.01
Ca	0.3	0.05
Cu	0.5	0.01
Fe	0.1	<0.01
Mg	0.1	0.05
Zn	3.0	<0.1

a. ^{68}Ga eluate

b. Ether extracted ^{68}Ga.

Table II

Trace Metals, $\mu g/100\ \mu l$, In ^{68}Ga Elutions, 1.0 N HCl, SnO_2 Column

	Samples			
Element	A	B	#3	#6
Al	0.02	0.02	0.02	0.02
→Ca	0.03	0.3	0.3	0.05
Cr	<.01	<.01	<.01	<.01
→Cu	<.01	0.1	0.1	<.01
→Fe	<.01	0.02	0.05	<.01
→Mg	0.01	0.03	0.03	0.02
Sn	<.1	<.1	<.1	<.1
→Zn	<.1	0.2	0.2	<.1

A. HCl purified on AG1X8 – less Fe, Mg, Cu, Zn
B. HCl reagent grade
3. ^{68}Ga Elution with solvent B
6. ^{68}Ga Elution with solvent A less Ca, Cu, Fe, Zn

TABLE III

Gallium-68 Platelet Labeling

Ga-68		Ligand		Platelets*				After Saline		
Pure	Vol(ml)	Type	Conc.(μg)	Type	Conc.×10^9	Media	Initial Label(%)	Wash(%)	Buffer	pH
EE†	0.5/6.0	oxine	200	rab	1.0	ACD:Sal	26	19	AC	6.8
EE	0.5/6.0	oxine	400	rab	1.9	ACD:Sal	84	50	AC	6.8
EE	0.5/6.0	oxine	400	rab	3.9	ACD:Sal	59	32	AC	6.8
EE	0.5/6.0	oxine	400	rab	1.9	ACD:Sal	42	23	AC	7.0
EE	0.5/6.0	oxine	200	hum	9.3	ACD:Sal	42	31	AC	7.0
EE	0.5/6.0	oxine	200	hum	9.3	PPP	19	6	AC	7.0
AG1×8	0.5	oxine	100	dog	--	ACD:Sal	10	--	OH^-	6.5
EE	0.5/6.0	Tro	40	dog	--	ACD:Sal	5	--	OH^-	7.4
EE	0.5/6.0	Tro	20	dog	--	ACD:Sal	12	--	OH^-	7.4
EE	0.5/6.0	Tro	60	dog	--	ACD:Sal	10	3	OH^-	7.4
EE	0.5/6.0	MPO	200	hum	2.3	Saline	63	56	OH^-	7.0
EE	0.5/6.0	MPO	200	hum	2.3	PPP	43	38	OH^-	7.0
EE	2.0	MPO	400	rab	6.5	Sal	45	35	AC	7.0
EE	2.0	MPO	400	rab	4.8	Sal	43	34	AC	7.0
EE	2.0	MPO	400	rab	12.3	Sal	49	43	AC	7.0

*Incubation 25-37°C, 15 min.
†EE - Ether extraction

TABLE IV

UPTAKE OF Ga–68 PLATELETS

% Injected Dose / Gram Tissue

	AORTA SCRAPED WHEN?	CAROTID ARTERIES	PROXIMAL AORTA	DISTAL AORTA	SPLEEN	HEART	LUNG	KIDNEY CORTEX	LIVER
RABBIT #1	20 minutes BEFORE Inj	0.03	0.03	0.11	4.65	0.06	0.13		0.23
RABBIT #2	Immediately BEFORE Inj	0.05	0.05	0.22	1.89	0.07	0.50	0.10	0.21
RABBIT #3	Immediately AFTER Inj	0.02	0.09	*4.88	10.24	0.11	0.44	0.10	0.28
RABBIT #4	Immediately AFTER Inj	0.02	0.04	0.13	3.02	0.03	0.31	0.05	0.33
RABBIT #5	Control NOT Scraped	0.04	0.04	0.06	6.06	0.14	1.30	0.08	0.28

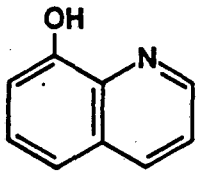
AVERAGE UPTAKE RATIO $\frac{\text{**Scraped distal aorta}}{\text{Normal proximal aorta}}$ = **3.77**

*Distal aorta on Rabbit #3 was VERY damaged

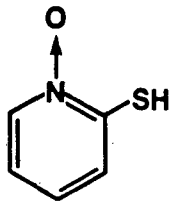
**Does NOT include distal aorta from Rabbit #3

Figure 1

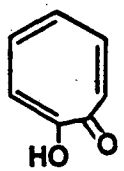
Ligands for Ga-68 Complex Formation



Oxine



Mercaptopyridine N-oxide



Tropolone

	Log Stability Constant		
	K_1	K_2	K_3
(a) Ga^{+3}	14.5	13.5	12.5
(b) In^{+3}	12.0	11.9	11.5

(a) L. Sillen, A. Martell, Stability Constants of Metal Ion Complexes, #25, 1971.
(b) C.J. Mathias, W.A. Heaton, M.J. Welch Intl. J. Appl. Radiat. Isot. 32, 651, 1981.

XBL 846-7161

Preparation and Labeling of DTPA-coupled Human Low-density Lipoproteins

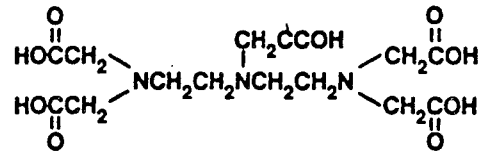
A. Isolation of hLDL[1]

Fresh human blood (0.1% EDTA, 5°C)

↓ Centrifuge 1200 rpm, 5°C, 25 min.

- R.B.C.
- Plasma[a]
  - I. Density: <1.019 Ultracentrifuge 40K rpm, 5°C, 18 hr
  - II. Density: 1.019-1.063 Ultracentrifuge 40K rpm, 5°C, 24 hr
    - I. → hVLDL + hIDL
    - II. → hLDL[a] (Nitrogen, 5°C)

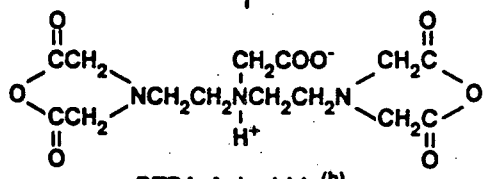
B. Synthesis of DTPA Anhydride[2]



DTPA

↓ I. Acetic anhydride/pyridine mix well, 65°C, 24 hr

↓ II. Filter, wash (acetic anhydride/ether), dry (50-60°C)



DTPA Anhydride[b]

[a]Contains EDTA, Merthiolate, Penicillin/Streptomycin as preservatives
[b]Verified by melting point and NMR; store in dessicator

(1) Nichols, A.V., Gong, E.L., Blanche, P.J., Forte, T.M. and Shore, V.G., Biochim. Biophys. Acta, *793*, 325-337 (1984).
(2) Hnatowich, D.J., Layne, W.W. and Childs, R.L., Int. J. Appl. Radiat. Isot., *33*, 327-332 (1982).

XBL 846-7153

Preparation and Labeling of DTPA-coupled Human Low-density Lipoproteins (cont.'d)

C. Coupling of DTPA to hLDL

hLDL soln. (7 μg/0.5 ml)

- I. Adjust to pH 7 with 0.5 M phosphate buffer, pH 6.5
- II. Add to 50-100 μg DTPA anhydride and incubate for 5 min., 25°C
- III. Sephadex G-50 (1 cm × 10 cm) 0.01 M Na_2HPO_4, 0.9% NaCl, pH 7.4
  - Free DTPA (remains on column)
  - hLDL-DTPA[a] (0.5 ml)

↓ 1 ml Ga-68$(OAc)_3$ incubate, 1 min., 25°C

hLDL-DTPA(Ga-68)[a]

D. Purification of Gallium-68

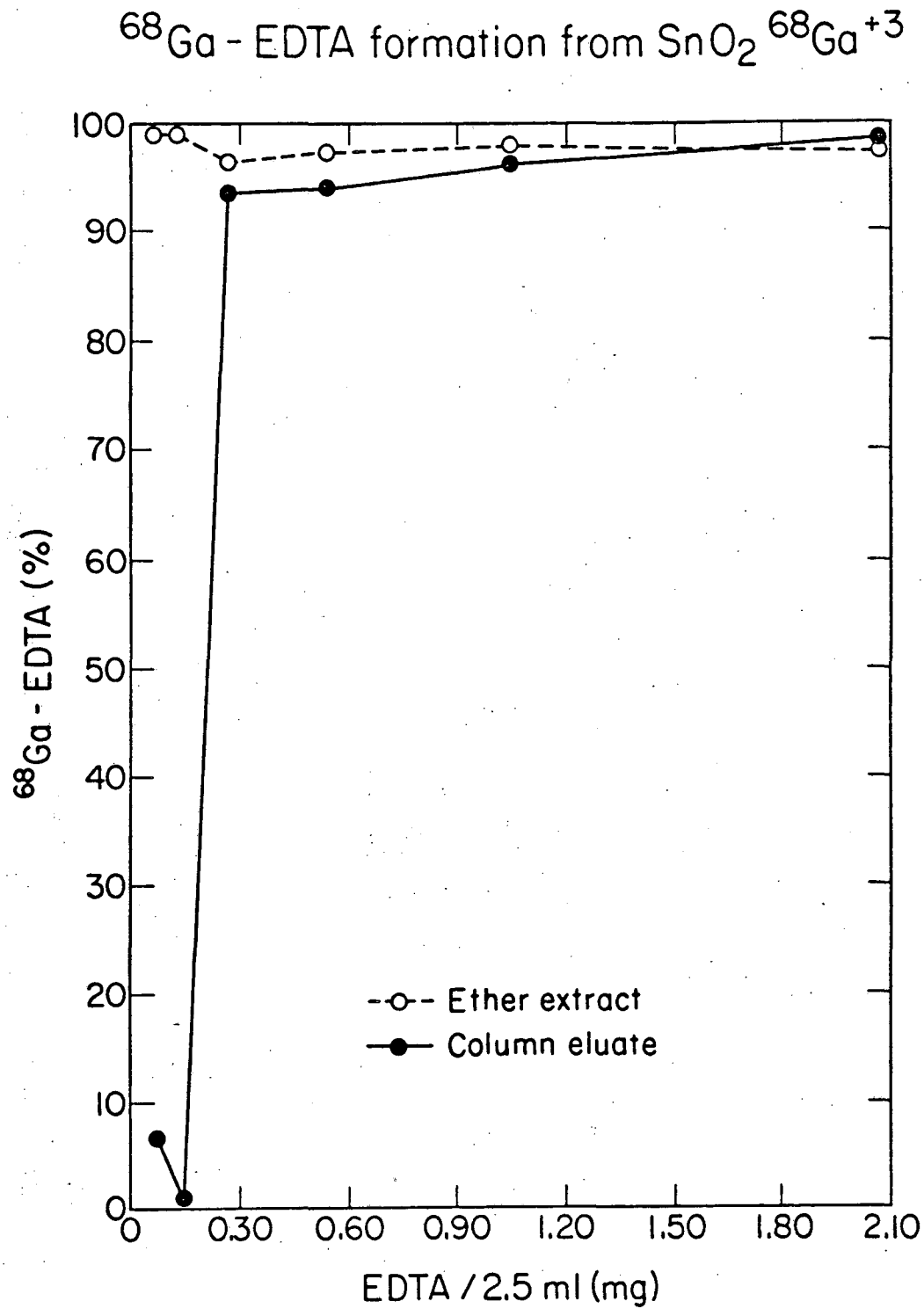
Extraction Apparatus[b]

- 2 ml Ga-68Cl_3, 1 N HCl eluate (generator)
- 2 ml 12 N HCl, resin purified
- 4 ml diethyl ether
  - Ethereal (Ga-68Cl_3)
    - I. Evaporate/N_2
    - II. 0.01 N HCl (0.5 ml), pH 2
    - III. 1 N NaOAc (0.5 ml), pH 6
    - ↓ Ga-68$(OAc)_3$
  - Aqueous (trace metal impurities)

[a]Analyzed by HPLC: BioGel TSK 50 (7.5 mm × 300 mm); 0.01 M Na_2HPO_4, 0.9% NaCl, pH 7.4
Ohno, Y., Okazaki, M. and Hara, I., J. Biochem. *89*, 1675-1680 (1981).
[b]MIXXOR liquid-liquid extraction system

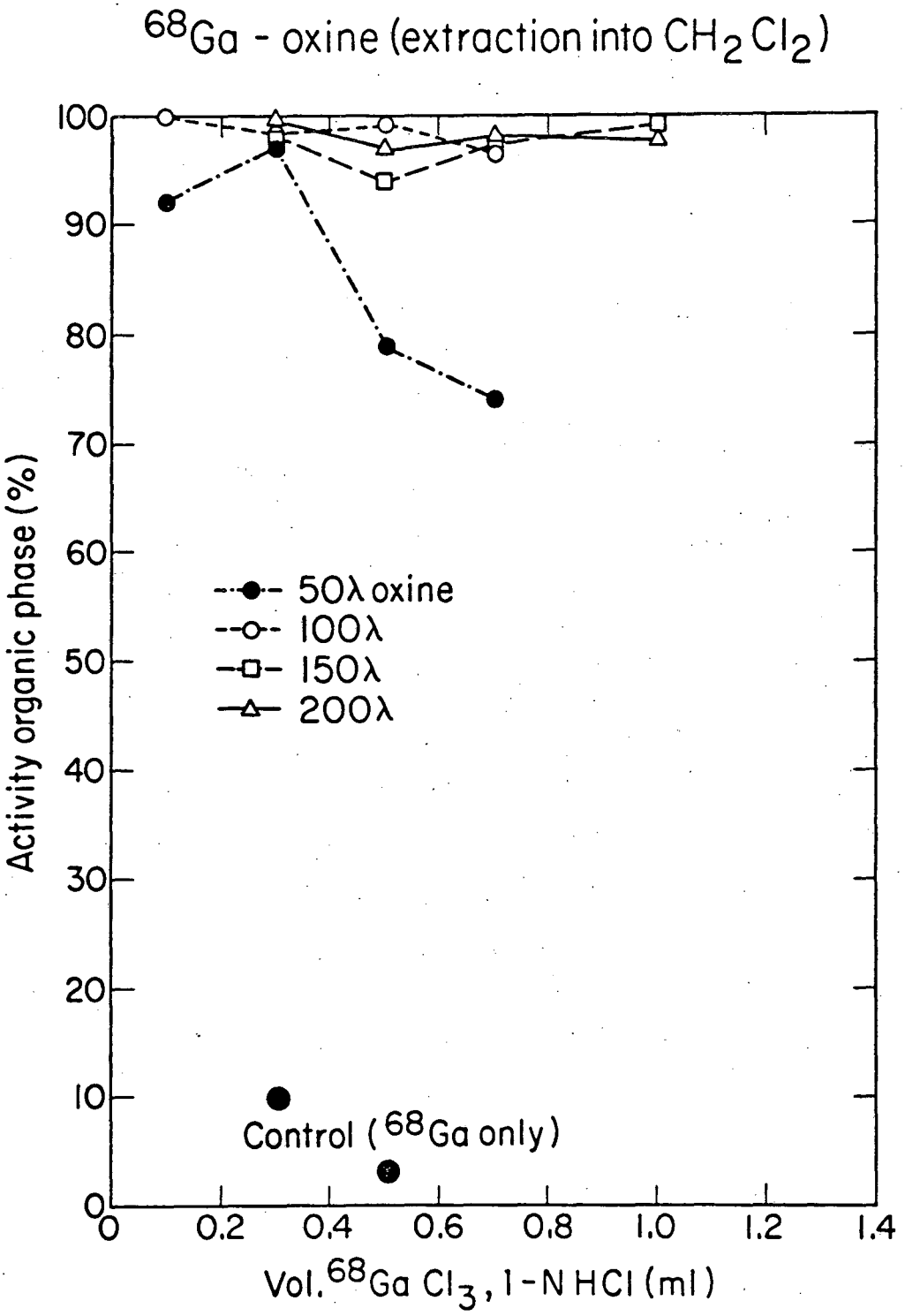
XBL 846-7154

Figure 3



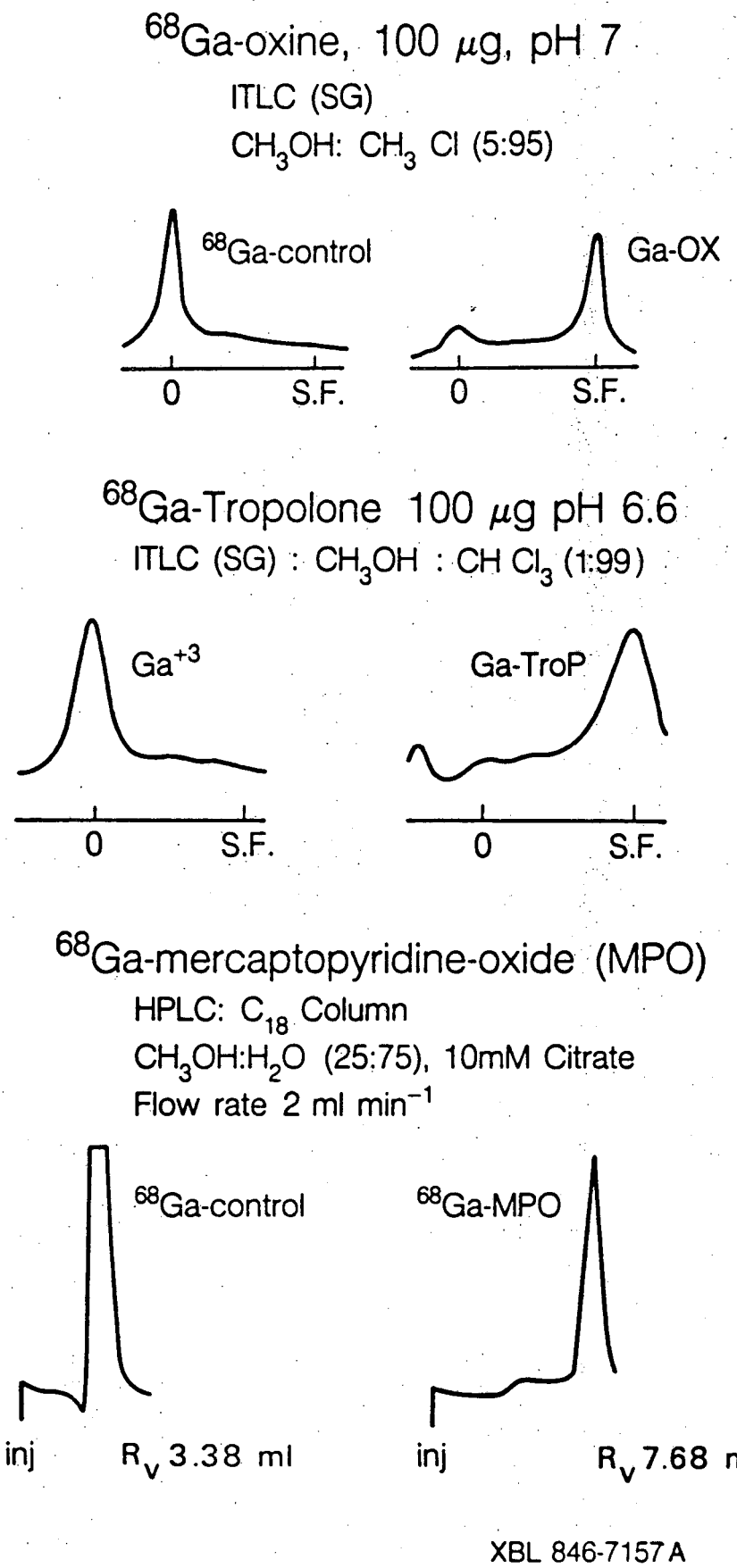
XBL 846-7141

Figure 4



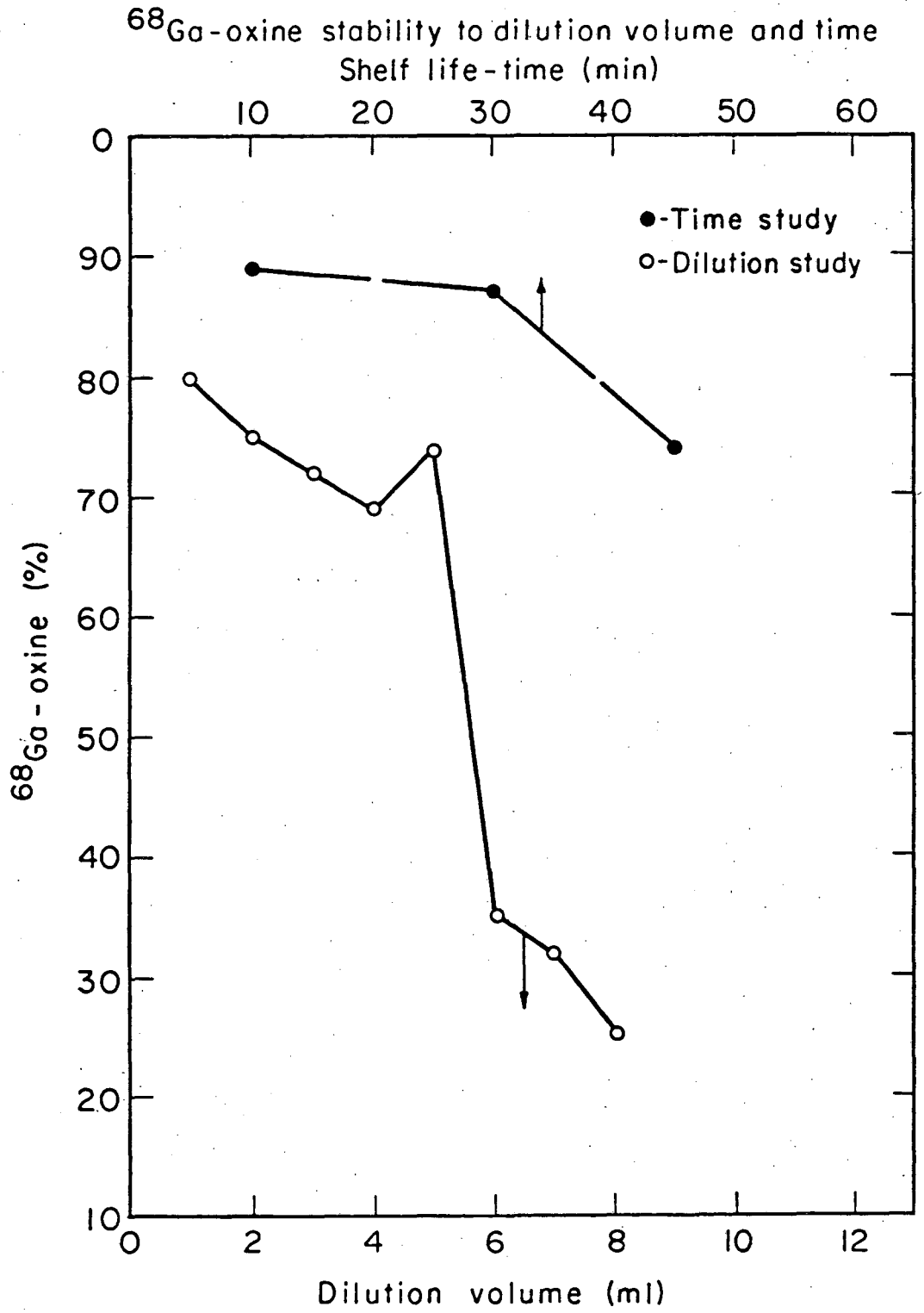
XBL 846-7142

Figure 5



XBL 846-7157A

Figure 6



XBL841-7546

Fig. 7

Preparation of Labeling of Platelets
with Gallium-68 MPO

A. Preparation of Ga-68 MPO

Ga^{68} (1N HCl)
↓
Ether extraction (via 6N HCl)
↓
Reconstitute in 0.5 ml of 0.01 N HCl (pH ≃ 2)
↓
Add 100-500λ, 0.5 M to pH 4-5.5 CH_3COO^- buffer
↓
Add 300λ–400λ MPO (1mg/ml)
| stir adjust pH to 7.0 (acid/base)
↓
Ga^{68}–MPO HPLC analysis
↓
Ga^{68}–MPO (≥ 90%)

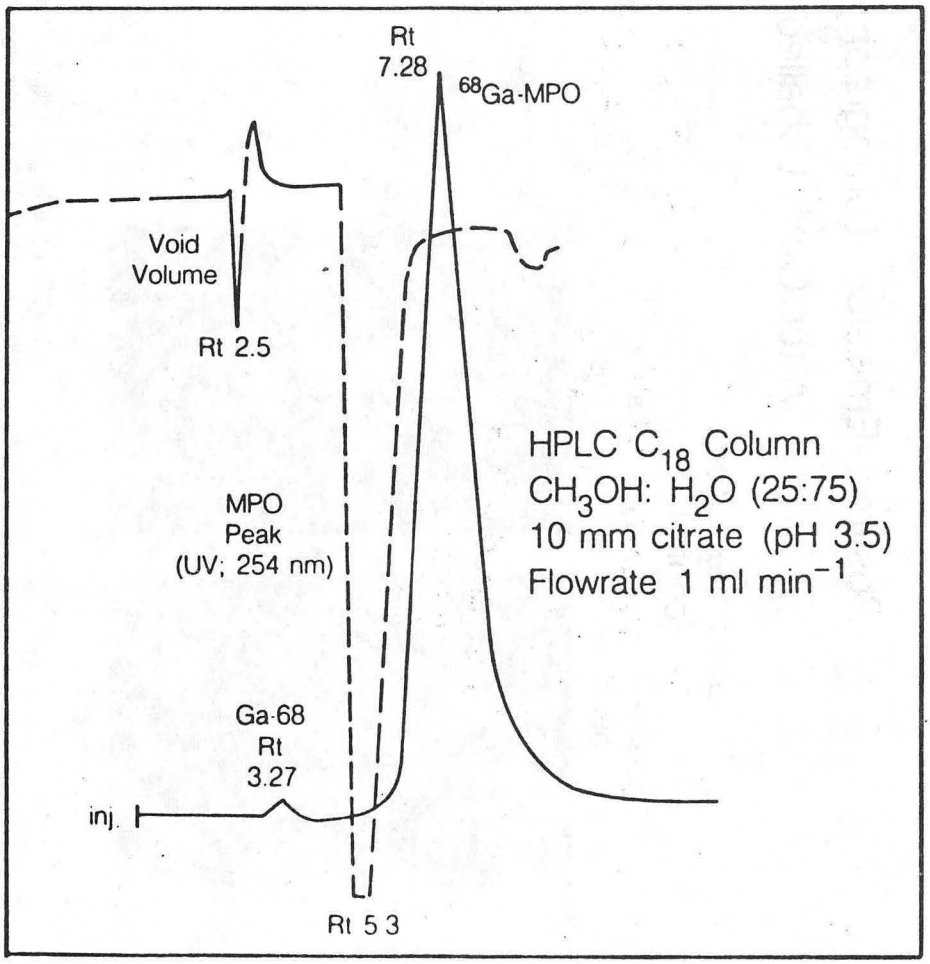
B. Platelet Preparation

Whole blood + ACD (anticoagulant)
Add saline (pH 7.0)
| centrifuge (200g, 200 min)
Pool PRP
| centrifuge (1100g, 20 min)
Platelet button
| saline (pH 7.0) wash
| centrifuge (1100g, 20 min)
Platelet button

C. Platelet Labeling

Add Ga^{68} MPO (A) to platelet button (B)
Incubate in 2-3 ml saline 15 min, Rt
Add saline (pH 7.0) to 10 ml
| centrifuge (1100g, 20 min)
Ga-68 MPO – platelet button
| saline (pH 7.0) wash
Ga-68 platelet button
Resuspend (normal saline)
/ \
Standards | Inject into recipient
↓
1 hr., 2 hr., 3 hr.
platelets survival study
↓
Imaging (PET)

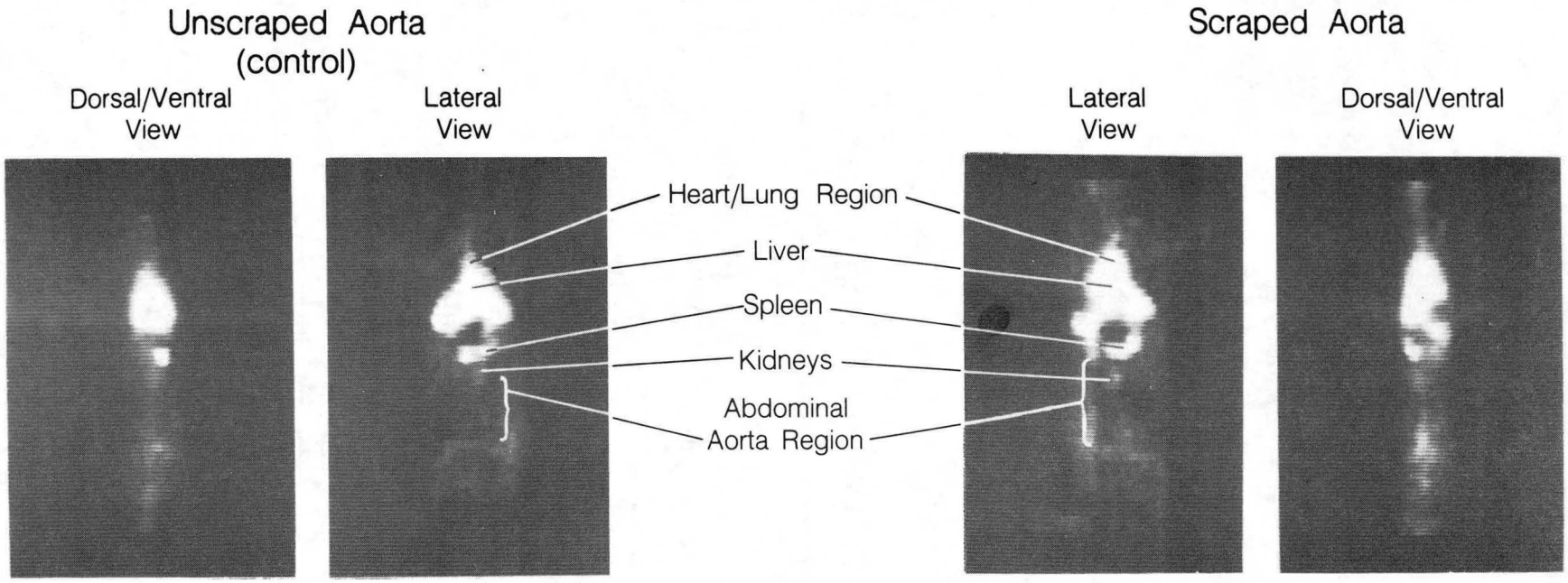
Ga^{68} – Mercaptopyridine-oxide (MPO)



XBL 8410-8022

Figure 8

Positron Emission Tomography Rabbit Whole Body Scans After Ga^{68} Labelled Platelets Injection

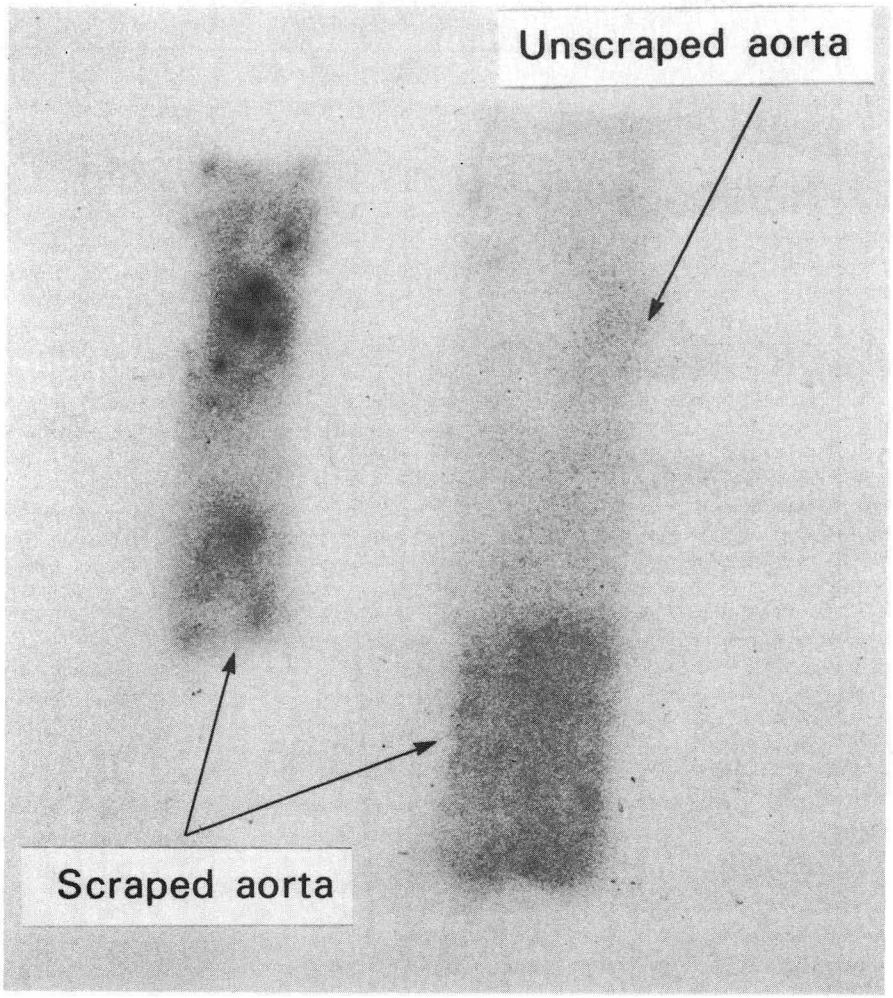


XBB 840-8739

60-70% Labelling Efficiency of Ga^{68} MPO onto Platelets
Final Platelet Suspension for Injection:

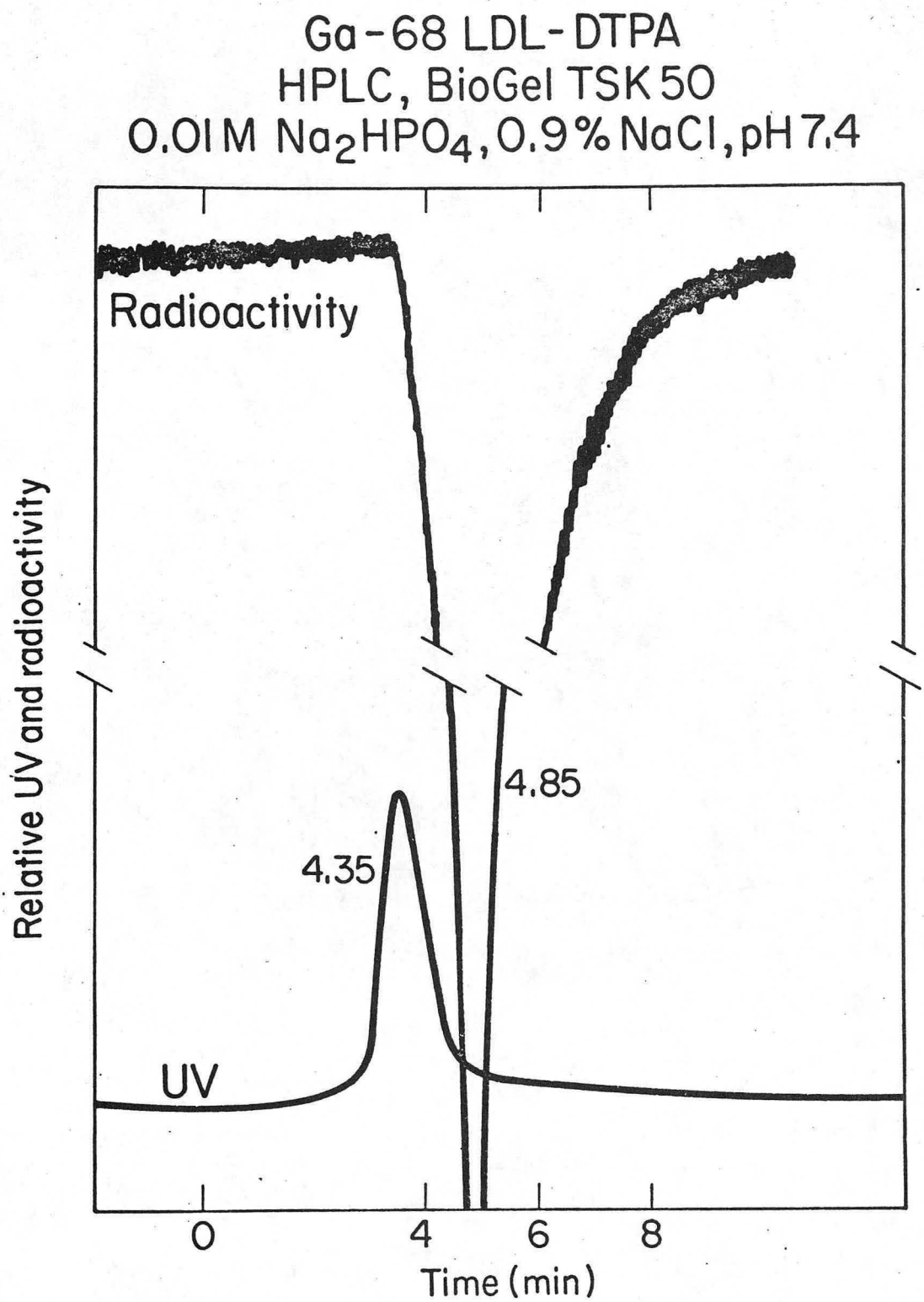
5-10 Million Platelets/cc
4-5 cc Injected into Rabbit
~1 mCi Ga^{68} activity

Figure 9



XBB 840-8869

Figure 10



XBL 845-7131

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

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