ORNL/TM--11043

DE89 009685

Contract No. DE-AC05-84OR21400

Health and Safety Research Division

NUCLEAR MEDICINE PROGRAM PROGRESS REPORT FOR QUARTER ENDING SEPTEMBER 30, 1988

F. F. (Russ) Knapp, Jr., Group Leader

K. R. Ambrose
A. P. Callahan
D. W. McPherson
P. C. Srivastava

J. F. Allred
S. L. Blystone
A. Kropp
E. C. Lisic
D. E. Rice
C. J. Rogers
G. Umbricht

Work sponsored by
DOE Office of Health and
Environmental Research

Date Published - March 1989

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37831
operated by
MARTIN MARIETTA ENERGY SYSTEMS, INC.
for the
U.S. DEPARTMENT OF ENERGY

MASTER

Previous reports in this series:

ORNL/TM-5809
ORNL/TM-5936
ORNL/TM-6044
ORNL/TM-6181
ORNL/TM-6371
ORNL/TM-6410
ORNL/TM-6638
ORNL/TM-6639
ORNL/TM-6771
ORNL/TM-6916
ORNL/TM-6958
ORNL/TM-7072
ORNL/TM-7223
ORNL/TM-7411
ORNL/TM-7482
ORNL/TM-7605
ORNL/TM-7685
ORNL/TM-7775
ORNL/TM-7918
ORNL/TM-8123
ORNL/TM-8186
ORNL/TM-8363
ORNL/TM-8428
ORNL/TM-8533
ORNL/TM-8619
ORNL/TM-8746
ORNL/TM-8827
ORNL/TM-8966
ORNL/TM-9037
ORNL/TM-9124
ORNL/TM-9343
ORNL/TM-9394
ORNL/TM-9480
ORNL/TM-9609
ORNL/TM-9707
ORNL/TM-9784
ORNL/TM-9937
ORNL/TM-10082
ORNL/TM-10238
ORNL/TM-10294
ORNL/TM-10377
ORNL/TM-10441
ORNL/TM-10618
ORNL/TM-10711
ORNL/TM-10839
ORNL/TM-11014

CONTENTS

SUMMARY

During this period the properties of the unknown metabolite released from Langendorff-perfused rat hearts administered radioiodinated 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP) have been further evaluated. Identification of this metabolite is important to provide a better understanding of the myocardial metabolism of methyl-branched fatty acids and to illuminate the factors affecting myocardial retention of such agents. The metabolite is the principal component in the outflow of the isolated rat hearts. Following isolation and purification of the metabolite, treatment with $NaBH_4$ had no effect on the chromatographic properties. In contrast, a much less polar product was formed by treatment with acetic anhydride, suggesting the presence of a primary or secondary hydroxyl group. In addition, the metabolite is soluble in dilute base and extracted from an acid solution with ether, demonstrating the presence of a carboxyl group. These combined results suggest BMIPP is metabolized to a hydroxy acid of unknown structure. Studies are now in progress to identify this material.

Studies of the effects of chain length on the complexation of a series of p-carboxyalkylphenylglyoxal bis-(N-alkylthiosemicarbazones) (TSC) have continued. After complexation with either Cu-64 or Cu-67 followed by activation to the tetrafluorophenyl esters, the bifunctional ligands were attached to BSA and purified by G-25 Sephadex. Yields varied from 2-3% to 40%, with higher yields for the shorter chain analogues. Because of simpler formation and higher yield, future studies will focus on the radiolabeling of antibodies with the short-chain analogues of the 1,2-diketone TSC derivatives.

Also during this period [I-131]IPPA was supplied to collaborators at the Institute of Clinical and Experimental Nuclear Medicine in Bonn, West Germany, for studies with an isolated working rat heart model.

EVALUATION OF METABOLITES FROM THE RADIOIODINATED 3-METHYL-BRANCHED FATTY ACID, 15-(p-IODOPHENYL)-3-R,S-METHYLPENTADECANOIC ACID (BMIPP)

The release of a polar component from Langendorff-perfused rat hearts injected with the monomethyl-branched iodophenyl fatty acid BMIPP has been described in earlier reports (ORNL/TM-10441). We have pursued the characterization of this polar metabolite from rat hearts as a means of understanding the factors affecting the slow myocardial washout of BMIPP observed in humans. Our earlier studies of this unidentified polar metabolite "X" have suggested the presence of both carboxyl and hydroxyl groups (ORNL/TM-10531). The unknown metabolite is considerably more polar (Table 1) than p-iodobenzoic acid (IBA) by thin-layer chromatography (TLC).

Table 1. Relative mobility (R_f) of various fatty acid analogues and metabolites by thin-layer chromatography (TLC) on silica gel G - PF 254.

	Solvent System	
Compound	Petroleum Ether:Ether: Acetic Acid (70:30:1)	Benzene:Dioxane: Acetic Acid (80:18:2)
BMIPP	0.30	0.60
p-Iodobenzoic acid (IBA)	0.25	0.40
"X"	0.15	0.35
"X" after $NaBH_4$ treatment	0.15	0.35
"X" after acetic anhydride treatment	0.35	0.65

During this quarter, further characterization of "X", isolated from the effluents of the Langerdorff hearts, was pursued. Following the procedures described previously (ORNL/TM-10441), isolated rat hearts were injected with [I-123]- or [I-131]-labeled BMIPP. Effluents from 3-15 min post-injection were combined, and the lipids extracted by an acidified Folch technique. Analysis by TLC confirmed the presence of "X" as the major radioactive component in the combined extracts, and the metabolite was then purified by silica gel column chromatography. Although the mass of the metabolite from these studies was not large enough for spectroscopic studies, simple chemical manipulations were conducted involving treatment with sodium borohydride ($NaBH_4$) and acetic anhydride. A more polar product on TLC analysis would be expected upon reduction of a keto function. Although there was no change in polarity following treatment with $NaBH_4$, treatment with acetic anhydride formed a nonpolar product (Table 1), suggesting that esterification of a primary or secondary hydroxyl group had occurred. Our studies are now focussed on obtaining sufficient mass of "X" by isolation of the perfusate lipids from multiple rat hearts administered low specific activity BMIPP to obtain enough material for derivatization and mass spectral analysis. The synthesis of β-hydroxy-BMIPP, the intermediary metabolite that would be expected to be formed by the usual β-oxidative pathway, is also being pursued.

In vivo studies were also performed to determine whether the "X" metabolite is also found in the plasma of animals injected with [I-125]BMIPP. Female Fischer-344 rats were injected intravenously, and blood samples were taken at 2, 5, and 30 min after injection. The plasma was separated from the red blood cells by centrifugation, and acidified Folch extracts of the plasma samples were obtained. TLC analyses of the plasma lipid extracts demonstrated that the majority of radioactivity at 2 and 5 min post-injection chromatographed with the BMIPP standard. At 30 min, however, a significant amount of radioactivity chromatographed in the region of "X" (Table 2).

Table 2. Percentage of radioactivity in rat plasma lipids chromatographing with BMIPP or "X" on TLC analysis.

	% Total activity*	
Time after injection (min)	"X"	BMIPP
2	<1	94 (93-95)
5	16 (11-22)	52 (44-60)
30	48 (37-56)	5 (4-6)

*Mean (with range) of four samples for 2 min and 30 min groups, and two samples for 5 min group.

Confirmation of the identity of this metabolite was performed by chromatographing [I-131]-labeled "X" isolated from the Langendorff heart effluents together with the [I-125]-labeled polar component from plasma. These studies show that despite the 3-methyl group, BMIPP is metabolized by rat hearts both in vivo and in the isolated heart system. Furthermore, the slow myocardial washout of BMIPP in humans may represent the loss of a similar metabolite.

BIFUNCTIONAL CHELATING AGENTS FOR COPPER AND RHENIUM LABELING OF MONOCLONAL ANTIBODIES

Monoclonal antibodies labeled with radioisotopes are important tools for both cancer diagnosis and treatment. Because copper-64 is one of the few long-lived positron emitters (12.7 h), our interest has focused on the possible use of copper-64 for labeling tumor-specific antibodies for imaging by positron emission tomography (PET). The attractive feature for use of copper-64 is the usual prolonged period between administration and imaging (24-48 h) required to allow vascular clearance and tumor specific uptake. Another radioisotope of copper which is of interest is copper-67 for the treatment of cancer. A new bifunctional chelating agent, p-carboxyethylphenylglyoxal-di-(N-methylthiosemicarbazone), has been reported for the labeling of monoclonal antibodies with technetium-99m. The chelation of copper to thiosemicarbazones (Figure 1) is well established and has been shown to form a stable complex under physiological conditions. The goal of our study is to explore the binding of copper to this type of bifunctional chelating ligand for the purpose of radiolabeling monoclonal antibodies.

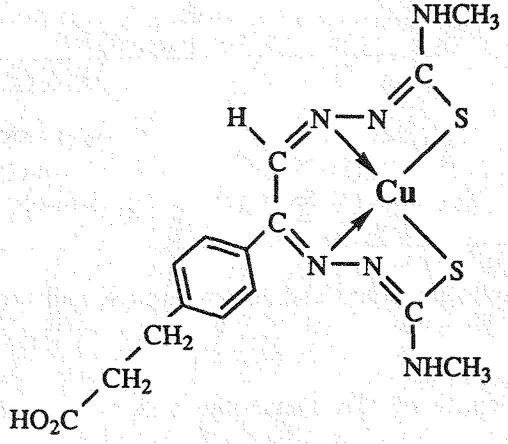
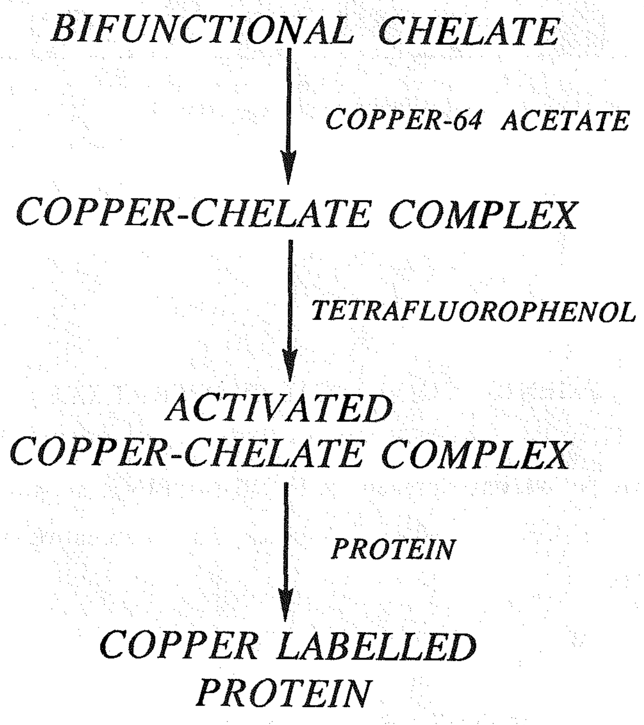


Figure 1.

We have reported the synthesis of a series of p-carboxyalkylphenylglyoxal-di-(N^4-methylthiosemicarbazone) analogues via an improved synthetic route (ORNL/TM-11014). We are currently utilizing these bifunctional chelating ligands to label a model protein to determine the best candidate for the monoclonal antibody labeling study. The methodology which we are pursuing for labeling the protein involves activation of the acid functionality by the use of tetrafluorophenol shown in Scheme I. Copper-64 or 67 as $CuCl_2$ is reacted with the ligand at pH 3.1 for 15 min at 70°C. The copper-ligand complex is esterified by the addition of tetrafluorophenol in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide. After purification, the activated copper-ligand complex is then coupled to the protein at pH 9.0 and purified by passage through a G-25 Sephadex column. The yield of the labeling of the protein using this approach varies from 2.5% to 40% (Figure 2) with a higher yield obtained for the shorter alkyl chain analogues. We are continuing our studies to determine the best candidate for radiolabeling model monoclonal antibodies with radioisotopes of copper.



Scheme I.

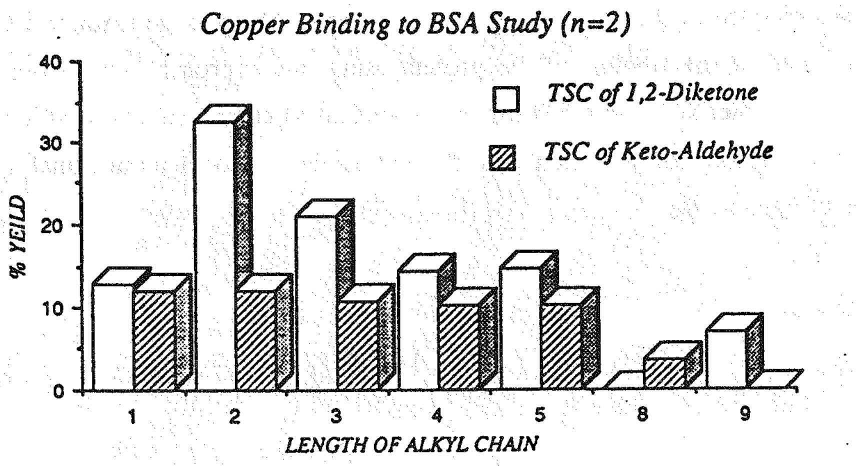


Figure 2.

AGENTS FOR MEDICAL COOPERATIVES

During this time period, one shipment of I-131[BMIPP] was supplied to the University of Bonn, West Germany, (Drs. J. Kropp and H.-J. Biersack) for collaborative studies with an isolated working rat heart system.

AGENTS PREPARED FOR COST-RECOVERY THROUGH THE ORNL ISOTOPES DISTRIBUTION OFFICE

One shipment of Pt-195m-labeled cis-dichlorodiammineplatinum(II) (cis-DDP) was supplied to Beth Israel Hospital, Boston, Massachusetts, (Dr. Kolodny) on a cost-recovery basis.

OTHER NUCLEAR MEDICINE GROUP ACTIVITIES

New Staff

Joachim Kropp, M.D., joined the Nuclear Medicine Program as a visiting scientist for a six-month period in August. Dr. Kropp is board-certified in nuclear medicine and is on the staff of the Institute for Clinical and Experimental Nuclear Medicine at the University of Bonn, West Germany. Dr. Kropp has extensive experience in clinical applications of iodine-123-labeled fatty acids using SPECT and pursued studies with us at ORNL on the metabolism of various radioiodinated fatty acids in an isolated rat heart system.

Publications

A. Hotze, B. Briele, F. Wolf, H. J. Biersack, and F. F. Knapp, Jr. "Localization and Activity of Inflammatory Bowel Disease Using Indium-111 Leucocyte Imaging," Nuklearmedizin, 27: 83-86 (1988).

W. J. Rzeszotarski, D. W. McPherson, J. W. Ferkany, W. J. Kinnier, L. Noronha-Blob, and A. Kirkien-Rzeszotarski "Affinity and Selectivity of the Optical Isomers of 3-Quinuclidinyl Benzilate and Related Muscarinic Antagonists," J. Med. Chem., 31: 1463-1465 (1988).

P. C. Srivastava, F. F. Knapp, Jr., and C. D. Pruitt "Potential Cerebral Perfusion Agents. Synthesis and Evaluation of a 1,4-Disubstituted Dihydropyridine Analogue," J. Het. Chem., 25: 667-669 (1988).

Patents

F. F. Knapp, Jr., and M. M. Goodman "Radiolabeled Dimethyl-Branched Long Chain Fatty Acids for Heart Imaging," U.S. Patent 4,764,358, Patent Gazette, p. 1331, August 16, 1988.

P. C. Srivastava, and F. F. Knapp, Jr. "Precursors to Radiopharmaceutical Agents for Tissue Imaging," U.S. Patent 4,764,598, Patent Gazette, p. 1393, August 16, 1988.

Papers

Several abstracts were authored and co-authored by members of the Nuclear Medicine Group at the 7th International Symposium on Radiopharmaceutical Chemistry held in Groningen, the Netherlands, on July 4-8, 1988:

C. Brihaye, M. Guillaume, F. F. Knapp, Jr., S. Dewez, D. E. Rice, and A. P. Callahan "Neutron Production of Osmium-191 and Separation from Iridium-192 For a Medical Osmium-191/Iridium-191m Generator."

M. M. Goodman and F. F. Knapp, Jr. "Radiochemical Synthesis of [^{18}F]-3-Methyl-Branched Omega Fluoro-Fatty Acids."

P. C. Srivastava and J. F. Allred "Synthesis and Biodistribution of Para-Iodophenyl Analogue of a Naturally Occurring Imidazole Ribonucleoside."

P. C. Srivastava, F. F. Knapp, Jr., J. F. Allred, and D. J. Buchsbaum "Evaluation of N-(p-[^{125}I]Iodophenyl)maleimide for Labeling Monoclonal Antibodies."

Members of the Nuclear Medicine Program co-authored two papers presented at the recent Annual Meeting of the European Society of Nuclear Medicine held in Milan, Italy, on August 26-September 2, 1988:

P. R. Franken, A. Dobbeleir, H. R. Ham, C. Brihaye, M. Guillaume, F. F. Knapp, Jr., and J. Vandevivere "Serial Iridium-191m Left Ventricular First Pass Angiocardiography and Thallium-201 Perfusion Imaging During Exercise in Patients with Coronary Artery Disease."

P. R. Franken, A. Dobbeleir, H. R. Ham, C. Brihaye, M. Guillaume, F. F. Knapp, Jr., and J. Vandevivere "First Pass Left Ventricular Ejection Fraction Using Iridium-191m from a New Carbon-Based Osmium-Iridium Generator System."

Miscellaneous

P. C. Srivastava was appointed to serve as a member of the Developmental Therapeutics Contracts Review Committee, National Cancer Institute (NCI), funded by a Scientific Review and Evaluation Award, for the term beginning July 1, 1988, to June 30, 1992. The committee is advisory to the Director of the National Cancer Institute (NCI) and has primary responsibility for the Division of Cancer Treatment.

Following participation in the Seventh International Symposium on Radiopharmaceutical Chemistry in Groningen, the Netherlands, July 4-8, 1988, F. F. Knapp, Jr. visited and coordinated collaborative research at the Institute for Clinical and Experimental Nuclear Medicine in Bonn, West Germany, the Department of Nuclear Medicine in Aachen, West Germany, the Cyclotron Research Center in Liege, Belgium, and the Cardiology Department at the Free University Hospital in Amsterdam, the Netherlands.

On September 9, 1988, P. C. Srivastava presented an overview of ORNL nuclear medicine research and discussed plans for collaborative interaction with the ORNL Nuclear Medicine Program on the development of radiopharmaceuticals for positron emission tomography at a meeting of the advisory committee for the University of Tennessee Hospital "Institute for Biomedical Imaging."

The Federal Laboratory Consortium for Technology Transfer has selected a description of the Osmium-191/Iridium-191m radionuclide generator system developed in the ORNL Nuclear Medicine Program as a presentation entitled "Safer Method for Diagnosis of Heart Disease - A New Radionuclide Delivery System." This presentation will be made as part of a general technology transfer seminar at a number of institutions throughout the U.S. over the next year.

ORNL/TM-11043

INTERNAL DISTRIBUTION

1. J. F. Allred
2. K. R. Ambrose
3. S. L. Blystone
4. T. A. Butler (Consultant)
5. A. P. Callahan
6. K. F. Eckerman
7. A. S. Garrett, Jr., M.D.
8. W. R. Garrett
9. M. M. Goodman (Consultant)
10. A. R. Hawthorne
11. G. W. Kabalka (Consultant)
12. S. V. Kaye
13. S. J. Kennel

14.-18. F. F. Knapp, Jr.

19. E. C. Lisic
20. D. W. McPherson
21. D. Pruett
22. G. Prosser
23. D. W. Ramey
24. D. E. Rice
25. C. R. Richmond
26. C. J. Rogers
27. P. C. Srivastava

28.-30. Central Research Library

31. Document Record Section

32.-33. Laboratory Records Dept.

34. Lab. Records, ORNL RC
35. ORNL Patent Section

EXTERNAL DISTRIBUTION

36. S. J. Adelstein, M.D., Shields Warren Radiation Lab., Boston, MA 02115.
37. H. L. Atkins, M.D., Radiology Dept., State Univ. of New York, Stony Brook, NY 11794
38. R. J. Baranczuk, Biomedical Products, 7899 Mastin, Overland Park, KS 66204
39. H. J. Biersack, M.D., Director, Institut fur Klinische und Experimentelle Nuklear Medizin Der Universitat Bonn, Sigmund Freud Strasse 25, 5300 Bonn 1, West Germany
40. C. Brihaye, Centre de Recherches du Cyclotron, Universite de Liege, Belgium
41. A. B. Brill, M.D., Ph.D., Commonwealth of Massachusetts, Univ. of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA 01605
42. T. F. Budinger, M.D., Donner Lab., LBL, Berkeley, CA 94720
43. W. Burr, M.D., Medical and Health Sciences Division, ORAU, Oak Ridge, TN 37831
44. P. Cho, OHER, U.S. DOE, MS-ER-73, Washington, D.C. 20545
45. D. W. Cole, Jr., U.S. DOE, ER-73, GTN, Washington, D.C. 20545
46. J. Crook, M.D., Ph.D., Medical and Health Sciences Division, ORAU, Oak Ridge, TN 37831
47. R. F. Dannals, Division of Nuclear Medicine, Johns Hopkins Medical Institutions, Baltimore, MD 21205
48. R. Dudczak, M.D., Dept. Nuclear Medicine, I. Medizinische Universitatsklinik, A-1090 Wien, Lazarettgasse 14, Vienna, Austria
49. J. Dunn, Mallinckrodt, Inc., 675 McDonnell Blvd., P.O. Box 5840, St. Louis, MO 63134
50. D. R. Elmaleh, Physics Research Dept., Massachusetts General Hospital, Boston, MA 02114
51. L. Feinendegen, Institut fur Medizin, Postfach 1913, D-5170, Julich 1, West Germany
52. A. Fritzberg, NeoRx Corporation, 410 West Harrison, Seattle, WA 98119
53. D. M. Goldenberg, M.D., Center of Molecular Medicine and Immunology, 1 Bruce Street, Newark, NJ 07103
54. M. Guillaume, Chef de Travaux, Centre de Recherches du Cyclotron, Universite de Liege, Belgium
55. D. R. Hamilton, Nuclear Medicine Branch, OTA/NCDRH/FDA, Rockville, MD 20857

56. C. J. Hardy, Chief, Isotope Division, Australian Nuclear Science and Technology Organization, Private Mail Bag 1, Menai, N.S.W. 2234, Australia
57. J. Hiltunen, Technical Research Centre of Finland, Reactor Laboratory, Otakaari 3 A, SF-02150 Espoo, Finland
58. K. Hubner, M.D., Department of Radiology, UT Memorial Hospital, Knoxville, TN 37920
59. K. J. Irgolic, Chemistry Dept., Texas A&M University, College Station, TX 77840
60. A. Jones, Dept. of Radiology, Harvard Medical School, Boston, MA 02115
61. G. Kirsch, Universite de Metz, Metz, France
62. J. Kropp, M.D., Institute for Clinical and Experimental Nuclear Medicine, University of Bonn, Bonn, West Germany
63. D. E. Kuhl, M.D., Division of Nuclear Medicine, University of Michigan Hospitals, University Hospital BIG 412/0028, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-0028.
64. S. Larson, M.D., Sloan-Kettering Inst. for Cancer Research, New York, NY 10021
65. J. Logic, M.D., University of Alabama Medical Center, Birmingham, AL 35233
66. H.-J. Machulla, Institut fur Med. Strahlenphysik, Universitats-klinikum, Hufelandstrasse 55, D-4300, Essen 1, West Germany
67. J. N. Maddox, DOE-OHER, ER-73, Washington, DC 20545
68. D. Moody, Group INC-11, MS J-519, LASL, Los Alamos, NM 87545
69. Office of Assistant Manager for Energy Research and Development DOE-ORO, Oak Ridge, TN 37831
70. K. J. Panek, Mallinckrodt Diagnostica (Holland) B.V., Westerduinweg 3, P.O. Box 3, 1755 ZG Petten, Holland
71. R. Patterson, M.D., Nuclear Cardiology, Crawford Long Hospital, 550 Peachtree Street, NE, Atlanta, GA 30365-2225
72. C. L. Partain, M.D., Director, Medical Imaging Division, Vanderbilt University Medical Center, Nashville, TN 37232
73. R. C. Reba, M.D., George Washington Univ. Med. Center, Washington, DC 20037
74. S. N. Reske, M.D., Nuklear Medizin Abteilung, RWTH Aachen, Pauwelsstrasse 1, 5100 Aachen, West Germany
75. M. Robbins, Mallinckrodt, Inc., 675 McDonnell Blvd., P.O. Box 5840, St. Louis, MO 63134
76. J. S. Robertson, DOE-OHER, ER-73/GTN, Washington, DC 20545
77. M. P. Sandler, M.D., Chief, Nuclear Medicine Section, Vanderbilt University Medical Center, Nashville, TN 37232
78. F. Snyder, ORAU, Oak Ridge, TN 37831
79. P. Som, DVM, Medical Department, BNL, Upton, NY 11973
80. S. C. Srivastava, Bldg. 801, Medical Dept., BNL, Upton, NY 11973
81. A. Solomon, M.D., UT MRCH, Knoxville, TN 37920
82. H. W. Strauss, M.D., Nuclear Medicine Div., Massachusetts General Hospital, Boston, MA 02114
83-92. Office of Scientific and Technical Information, DOE, Oak Ridge, TN 37831
93. A. Vessey, Eastman Pharmaceuticals, 25 Great Valley Parkway, Great Valley, PA 19355
94. F. Visser, M.D., Cardiology Dept., Free University Hospital, De Boelelaan 117, Amsterdam, The Netherlands
95. H. N. Wagner, Jr., M.D., Div. of Nuclear Medicine, Johns Hopkins Medical Institutions, Baltimore, MD 21205
96. L. C. Washburn, Medical and Health Sciences Division, ORAU, Oak Ridge, TN 37831
97. A. P. Wolf, BNL, Upton, NY 11973
98. D. V. Woo, Hahnemann University, Philadelphia, PA 19102
99. R. W. Wood, Jr., DOE-OHER, Washington, DC 20545
100. S. Wynchank, Research Institute for Medical Biophysics (RIMB), Republic of South Africa