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Nuclear Medicine Technology Progress Report for Quarter Ending December 31, 1978

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OAK RIDGE NATIONAL LABORATORY
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NUCLEAR MEDICINE TECHNOLOGY PROGRESS REPORT FOR QUARTER ENDING DECEMBER 31, 1978

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OAK RIDGE NATIONAL LABORATORY
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ORNL/TM-5936

ORNL/TM-6044

ORNL/TM-6181

ORNL/TM-6371

ORNL/TM-6410

ORNL/TM-6638

ORNL/TM-6639

CONTENTS

SUMMARY

Of special interest in this report is the recent synthesis of ^{117m}Sn-labeled 12,12-dimethyl-12-stannahexadecanoic acid. Although tissue distribution studies with this potential myocardial imaging agent demonstrated only marginal heart uptake of radioactivity, these studies are important since they clearly demonstrate that tissue-specific ^{117m}Sn-labeled agents can be prepared. Because of the low reactor production costs and the attractive radionuclidic properties, we envision that ^{117m}Sn-labeled tissue-specific agents may be an exciting new class of radiopharmaceuticals. In the present report, we also illustrate the high-quality heart scans in rats that have been obtained following intravenous administration of ^{123m}Te-labeled 9-tellurahepta-decanoic acid.

In this progress report, we also describe continuing studies with ^{11}C and ^{195m}Pt. Additional patient studies with ^{11}C-DL-tryptophan and ^{11}C-1-aminocyclobutanecarboxylic acid (^{11}C-ACBC) at the Oak Ridge Associated Universities have further illustrated the usefulness of these agents for the positron emission tomographic imaging studies of the pancreas and tumor tissue, respectively. Studies of the reactor production of ^{195m}Pt by the Szilard-Chalmers process have been completed. Although studies in the Oak Ridge Research Reactor did demonstrate that the specific activity could be increased by irradiation of PtO_2 at the low flux of $\sim 10^{14}$ neutrons $cm^{-2}sec^{-1}$, similar experiments in the High Flux Isotope Reactor at $\sim 10^{15}$ neutrons $cm^{-2}sec^{-1}$ were not successful. These studies have demonstrated that the preparation of

high specific activity ^{195m}Pt by the Szilard-Chalmers process using this technique is impractical. Finally, continued studies with the KB tumor cell diffusion chamber technique have shown that a dose-response relationship can be demonstrated following injection of both cyclophosphamide and *cis*-dichlorodiammineplatinum (II). These results suggest that the diffusion chamber technique could be an important tool to study the uptake and effects on cell growth of radiopharmaceuticals and other agents.

CARBON-11

T. A. Butler

One ^{11}C production run was made this quarter for the Medical Cooperative Program with Oak Ridge Associated Universities (ORAU) to study the application of ^{11}C-labeled amino acids for tumor localization and pancreas imaging in human patients. Both ^{11}C-1-aminocyclobutane-carboxylic acid (^{11}C-ACBC) and ^{11}C-DL-tryptophan were prepared and were used to examine patients by positron emission tomography at ORAU. Carbon-11-tryptophan continues to show superiority over ^{11}C-DL-valine as a pancreatic imaging agent, and ORAU expects to complete a sufficient number of studies during the next quarter to demonstrate clearly this superiority.

PLATINUM-195*m*

J. D. Hoeschele and T. A. Butler

As part of the continuing Medical Cooperative Program to study platinum antitumor compounds, two shipments of ^{195m}Pt-labeled Na_2PtCl_6 were made to the University of Kentucky Medical Center and two shipments were made to the University of Southern California.

In cooperation with W. Wolf at the University of Southern California, we have completed a short-term study to determine the feasibility of enhancing ^{195m}Pt specific activity by the Szilard-Chalmers process. The experimental design outlined in the previous quarterly report (ORNL/TM-6639) was followed. Anhydrous PtO_2 (normal isotopic abundance) samples in powdered form were irradiated in the Oak Ridge Research Reactor (neutron flux $\sim 10^{14}$ neutrons $cm^{-2}sec^{-1}$) and the High Flux Isotope Reactor (neutron flux $\sim 10^{15}$ neutrons $cm^{-2}sec^{-1}$). The irradiated material was leached with successive portions of *aqua regia* at ambient temperatures ($\sim$20°C) and each leachate assayed for platinum and ^{195m}Pt content. We found a significant enhancement of ^{195m}Pt specific activity in the case of the low flux ($\sim 10^{14}$ neutrons $cm^{-2}sec^{-1}$) irradiation but none at the higher flux. Visual evidence suggested that gamma heating of the PtO_2 at $\sim 10^{15}$ neutrons $cm^{-2}sec^{-1}$ resulted in thermal decomposition of PtO_2 and annealing of any Szilard-Chalmers effect that may have been produced. Experimental data from the low flux ($\sim 10^{14}$ neutrons $cm^{-2}sec^{-1}$) experiment, normalized to a specific time are shown in Table 1.

Table 1. Results of acid leaching of PtO_2 irradiated at a flux of $\sim 10^{14}$ neutrons $cm^{-2}sec^{-1}$

Fraction	Specific activity, mCi/mg of Pt	Enhancement factor
Unleached PtO_2	0.056	
25-min leach	0.309	5.52
30-min leach	0.138	2.46
60-min leach	0.103	1.84
120-min leach	0.087	1.55

Our initial goal was to achieve a ^{195m}Pt specific activity enhancement factor of 5 which was demonstrated in the first leach solution.

Extrapolation of these data to a practical scale of ^{195m}Pt-labeled compound synthesis reduces the attractiveness of this method of enhancement of ^{195m}Pt specific activity. Current practice in the microscale synthesis of such labeled compounds requires a minimum of ~3 mg of platinum. In the first leach solution, where an enhancement factor of 5.52 was achieved, only 0.16% of the platinum target was dissolved, which indicates that 1.8 g of platinum in the form of PtO_2 powder would be needed for reactor irradiation to obtain 3 mg of platinum for subsequent chemical operations. To maximize the specific activity and radionuclidic purity of ^{195m}Pt, enriched (97.4%) ^{194}Pt target material is used for our reactor irradiations. Since enriched ^{194}Pt is not now available on the world market, it does not appear prudent to use our limited supply for this purpose.

TIN-117*m*

F. F. Knapp, Jr., T. A. Butler, K. R. Ambrose and D. V. Woo

A preliminary assessment of the factors influencing the reactor production of ^{117m}Sn by the $^{116}Sn(n, \gamma)^{117m}Sn$ nuclear reaction can be made on the basis of five irradiation experiments. All irradiations were made in the same physical location in the hydraulic tube facility of the High Flux Isotope Reactor at a neutron flux of $\sim 2.5 \times 10^{15}$ neutrons $cm^{-2}sec^{-1}$. The targets were enriched (95.74%) stable ^{117}Sn varying in weight from 10 to 73 mg, and the irradiation periods varied from 2 to 14 days. The ^{117m}Sn production yields are consistent with an effective cross section ($\bar{\sigma}$) of 13.1 millibarns and follow the radioisotope production equation $A = N \phi \sigma (1 - e^{-\lambda t})$, where A is the activity, N is the number of target atoms, ϕ is the neutron flux, σ is the neutron cross section, λ is the decay constant of the radionuclide produced, and t is the irradiation time. This equation holds for cases in which the number of target atoms does not decrease significantly and the radionuclide product disappears primarily by radioactive decay processes. We can conclude the ^{117m}Sn does not have a significant neutron capture cross section and that target self-shielding is not a significant factor. The maximum specific activity of ^{117m}Sn which we can achieve is calculated to be ~ 4.4 mCi/mg. In excess of 140 days of irradiation would be required to reach the maximum value [where $(1 - e^{-\lambda t}) \rightarrow 1$ as $t \rightarrow \infty$].

In the last quarterly report in this series (ORNL/TM-6639), we described the development of a microscale system for the preparation of

^{117m}Sn-labeled tin tetrachloride ($^{117m}SnCl_4$). Our interest in the preparation of ^{117m}Sn-labeled radiopharmaceuticals was stimulated because of the attractive properties of the radionuclide: (1) emission of a single 158 keV γ-photon in 87% abundance, (2) a 14-day physical half-life, (3) inexpensive reactor production [$^{116}Sn(n, \gamma)^{117m}Sn$], and (4) the low expense of the ^{116}Sn target material ($0.35/mg). These properties coupled with the versatility of organotin chemistry and the stability of many organotin compounds suggested that an exciting array of tissue-specific ^{117m}Sn-labeled agents could be prepared and that the synthesis of model agents should be explored. As an initial member of this potential new class of radiopharmaceuticals, we have chosen ^{117m}Sn-labeled 12,12-dimethyl-12-stannahexadecanoic acid. This compound was perfectly tailored for our development efforts since the preparation of the unlabeled fatty acid had been previously reported (S. B. Andrews, *et al.*, *Biochimica et Biophysica Acta*, 506, 1-17, 1978). In addition, the tin fatty acid was incorporated into the membrane lipids of *Acholeplasma laidawii*, indicating that such a compound was metabolically active. The possibility was considered that perhaps the tin fatty acid would also be metabolically active in mammalian systems, that it would be concentrated by the myocardium and that the ^{117m}Sn-labeled compound could therefore be used for heart imaging. The availability of the microscale procedure for the synthesis of $^{117m}SnCl_4$ has made possible the synthesis of ^{117m}Sn-labeled 12,12-dimethyl-12-stannahexadecanoic acid. The preparation of this compound is shown below. The ^{117m}Sn radionuclide is denoted as *Sn.

$$*Sn + Cl_2 \rightarrow *SnCl_4 + Me_4Sn \rightarrow Me_3*Sn\text{-}Cl + BuLi \rightarrow Me_3Bu*Sn +$$

$$Br_2 \rightarrow Me_2Bu*Sn\text{-}Br + LiAlH_4 \rightarrow Me_2Bu*Sn\text{-}H + CH_2{=}CH\text{-}(CH_2)_8\text{-}COOMe$$

$$\rightarrow Me_2Bu*Sn\text{-}(CH_2)_{10}\text{-}COOMe \rightarrow Me_2Bu*Sn(CH_2)_{10}\text{-}COOH$$

The $^{117m}SnCl_4$ was reacted with three molar equivalents of commercially available Me_4Sn to give, *via* the comproportionation reaction, the trimethyltin chloride ($Me_3*Sn\text{-}Cl$). Following subsequent alkylation with n-butyl lithium and methyl cleavage with one equivalent of bromine, the resulting dimethylbutyltin bromide was reduced to give the tin hydride ($Me_2Bu*Sn\text{-}H$). The hydride added in the expected Markownikoff fashion across the terminal olefinic moiety of methyl-10-undecenoate to give the ^{117m}Sn-labeled fatty acid methyl ester. A variety of absorptive column chromatographic systems were attempted for optimal purification of the radiolabeled methyl ester and 60-200 mesh neutral silicic acid was found to be an efficient system. The purified product showed only one radioactive spot when analyzed by thin-layer radiochromatographic analysis which co-chromatographed with methyl-12,12-dimethyl-12-stannahexadecanoate. Mass spectral analysis of this product, however, indicated an approximate 1:1 mixture of the methyl esters of 12,12-dimethyl-12-stannahexadecanoate and 12-methyl-12-butyl-12-stannahexadecanoate. These results indicate that the reaction did not proceed as smoothly as was anticipated, and that an alkyl-metal exchange had probably occurred. This reaction is presently being studied in more detail.

The radiolabeled methyl ester was converted to the free acid by

basic hydrolysis and complexed to 6% bovine serum albumin solution and then administered intravenously to female rats. The tissue distribution results (Fig. 1) indicate that radioactivity was not specifically localized in the heart tissue. The important results of these studies, however, is our demonstration that a biologically important molecule can be radiolabeled with ^{117m}Sn. Studies are now being initiated to determine the effects of total chain length and the position of the tin heteroatom on the heart uptake of ^{117m}Sn-labeled fatty acids.

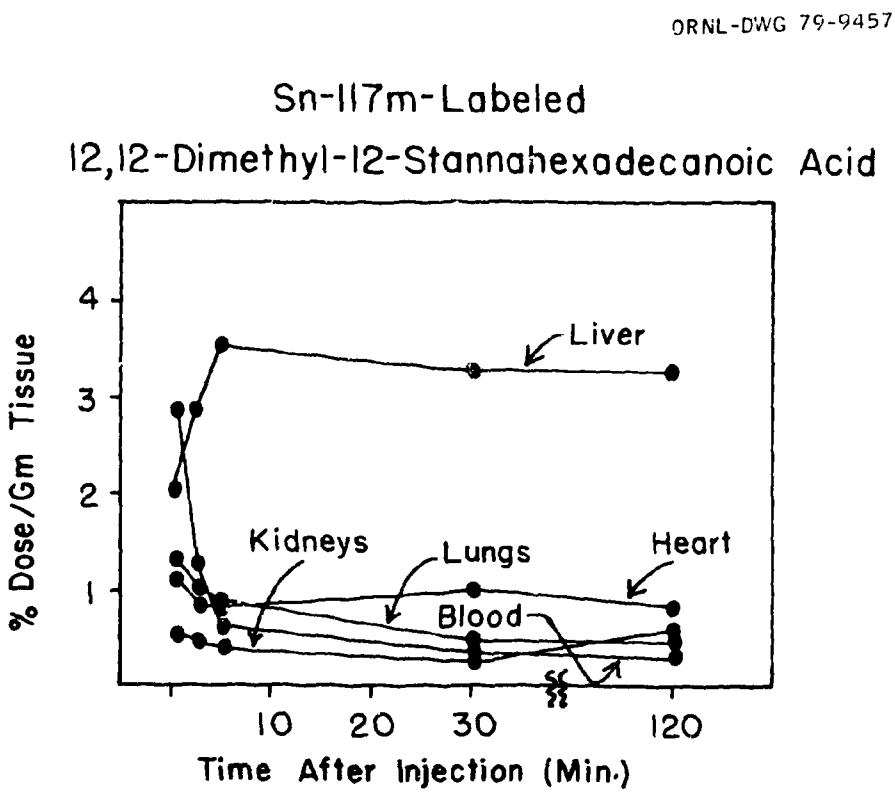

Fig. 1. Tissue distribution of radioactivity following administration of ^{117m}Sn-labeled 12,12-dimethyl-12-stannahexadecanoic acid.

TELLURIUM 123m

F. F. Knapp, Jr., and K. E. Ambrose

Our biological studies with ^{123m}Te-labeled 9-telluraheptadecanoic acid have continued. The results of tissue distribution experiments described in ORNL/TM-6638 indicated the pronounced heart uptake of this "isostere" of oleic acid, suggesting that ^{123m}Te-labeled 9-telluraheptadecanoic acid or similar compounds may represent a new class of potential myocardial imaging agents. We have now successfully imaged rat hearts with a small-animal rectilinear scanner following injection of this new agent (Fig. 2). The first scan of the rat was initiated 10 min after injection, and the heart appears as a hot spot above the liver mass. After 1 hr, the animal was sacrificed and the liver removed for the second scan where the heart is clearly visible. No other areas of concentration of radioactivity are observed in any other region of

ORNL PHOTO 0959-79

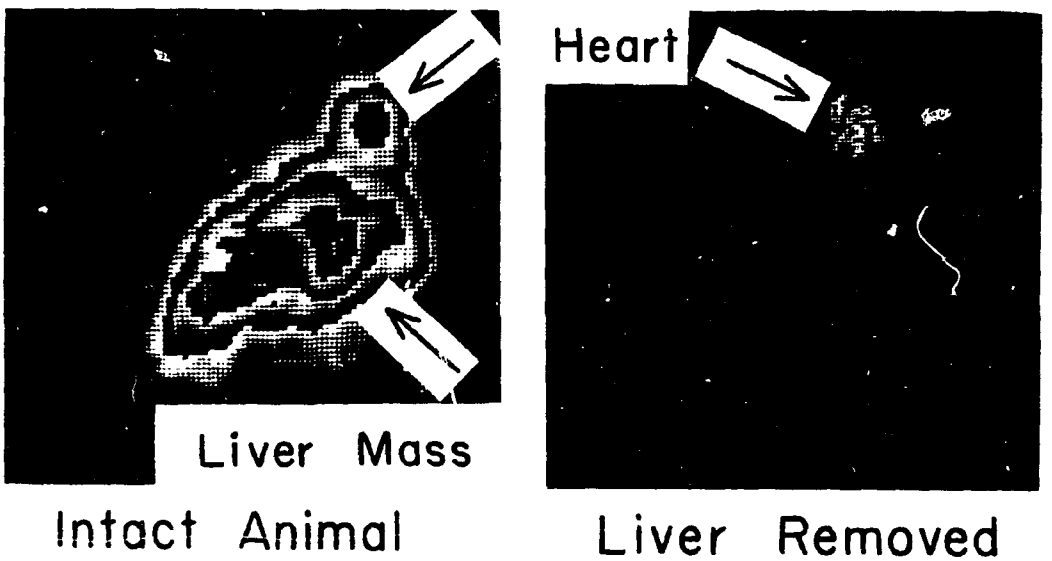

Fig. 2. Rectilinear scan of a female rat after administration of 50 μCi of ^{123m}Te-labeled 9-telluraheptadecanoic acid.

the animal carcass. In a parallel experiment, the animal was sacrificed after the initial scan and the heart removed and immediately perfused with saline to remove all of the blood from the cardiac chambers. The excised heart was scanned and showed an identical image to that observed in the scan of the intact animal. These results confirm the tissue distribution and preceding scanning data and demonstrate that the radioactivity was present primarily in the heart muscle and not in the blood pooled within the cardiac chambers. A rather intriguing result of our studies is the much longer residence time of the ^{123m}Te-labeled fatty acids in the heart tissue compared to the rapid clearance of alkanoic acids such as palmitic acid. These data may indicate that the ^{123m}Te-labeled agents are efficiently extracted by the myocardium and trapped in some storage form which is not further metabolized. Even if β-oxidation of these unusual fatty acids were initiated, it would seem improbable that this catabolic process could proceed any further than the immediate vicinity of the tellurium heteroatom.

Unfortunately, more recent longer term tissue distribution studies have demonstrated the toxicity of the telluro fatty acids. This toxicity is manifested by blood in the urine and in some cases the animals have died within two days after injection. The ^{123m}Te-labeled agents that have been studied have specific activities in the region of 10-20 mCi/mmole. We are now initiating more detailed toxicity tests to study further this problem. The apparent toxicity can possibly be overcome by using high specific activity material, and we are now particularly interested in the ^{75}Se-labeled fatty acids where much higher specific activities can be prepared. The potential use of

^{73}Se-labeled fatty acids for positron emission tomography of the heart is intriguing.

As a result of the apparent greater stability of the methyl esters of the (alkyl telluro)-substituted fatty acids, we also performed tissue distribution experiments in rats with ^{123m}Te-labeled methyl 9-telluraheptadecanoate. The results of these studies are illustrated in Fig. 3 and demonstrate only marginal uptake of radioactivity in heart tissue following intravenous administration of this compound.

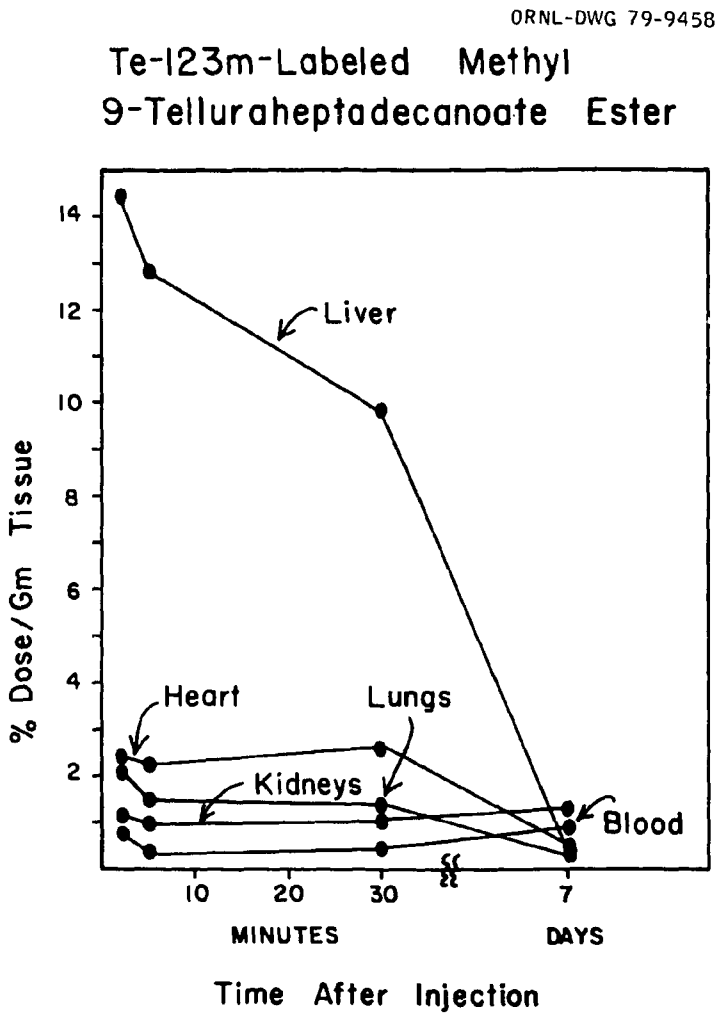

Fig. 3. Tissue distribution of radioactivity following administration of ^{123m}Te-labeled methyl-9-telluraheptadecanoate.

RECTILINEAR SCANNING SYSTEM

D. V. Woo

A rectilinear scanner interfaced to a dedicated PDP-8E computer (Digital Equipment Corporation) was recently installed within the facilities of the Nuclear Medicine Technology Group. This scanning system was developed by P. R. Bell and enables scans to be obtained with small animals after administration of radiopharmaceuticals. The computer utilizes the Oak Ridge Imaging System (ORIS; ORNL/TM-5875/V2) software package for image enhancement. A number of rat scans performed during this quarter have resulted in the clear delineation of various target organs. In particular, we have demonstrated significant myocardial uptake of various ^{123m}Te-labeled fatty acid analogs (*vide ante*). Quantitation of the radioactivity within the heart tissue was obtained by integration of the heart area of sequential scans after administration of ^{123m}Te-9-telluraheptadecanoic acid. The relative integral data closely parallel data obtained from invasive biodistribution studies with the same agent. These studies demonstrate the usefulness of this instrumentation to noninvasively obtain sequential concentration levels of radiopharmaceuticals and other radiolabeled agents. These data can be used to develop suitable kinetic models that allude to actual physiological events occurring within a given organ. These techniques can also be used to determine the relative bioavailability of a given radiopharmaceutical. Finally, operational procedures for the scanner and computer have been condensed during this quarter from more detailed documentation in order that routine scans can be performed easily by a trained technician.

DIFFUSION CHAMBER ASSAY SYSTEM

K. R. Ambrose

The diffusion chamber assay technique described in ORNL/TM-6639 was used to investigate further the *in vivo* antitumor effects of cyclophosphamide (CP) and *cis*-dichlorodiammineplatinum(II) (*cis*-DDP). Dose-response studies of the two agents were performed in order (1) to determine appropriate doses to use in future combinational drug studies and (2) to further characterize the chamber technique as an assay for cytotoxic or growth inhibitory effects of drugs. Diffusion chambers containing KB (human carcinoma) target cells were implanted into the peritoneal cavity of Fischer strain rats. One day later, the rats were injected with different doses of either CP, *cis*-DDP or saline. Chambers were removed 48 hr after injection, and the cells within the chambers were counted microscopically using trypan blue dye to establish cell viability. Because of the time required for microscopic cell enumeration, experiments were limited to 5 groups (4 test + control) with 10 chambers per group. The statistical significance of the differences between the mean cell counts in each group was analyzed by the Wilcoxon two-sample test, a simple nonparametric statistical test.

The results of four dose-response studies using CP or *cis*-DDP are shown in Tables 2 and 3. In general a decreased dosage of drugs resulted in decreased inhibition of tumor cell growth. With the exception of test groups 5 and 10 mg CP/kg, analysis of the mean cell counts by the Wilcoxon test showed all test groups to be significantly different from the control groups at the 95% confidence level (most were significant at the 99.9% confidence level). The majority of test dose groups also

differed significantly from their neighboring dose levels at the 95% confidence level. The reproducibility of the diffusion chamber assay can be seen in the overlap of data points in experiments shown in Tables 2 and 3 and in other studies where similar dosages were used.

Table 2. Inhibition of KB tumor cell growth in implanted diffusion chambers after injection of cyclophosphamide (CP)

	Inhibition of cell growth[a]%	
Dosage of CP (mg/kg)	Experiment 18-78	Experiment 27-78
75	89	
50	83	83
25	70	70
10		11
5		10

[a]Percent inhibition cell growth = (1 - mean No. cells in test group/mean No. cells in control group x 100).

Table 3. Inhibition of KB tumor cell growth in implanted diffusion chambers after injection of *cis*-dichlorodiammineplatinum(II) (*cis*-DDP)

	Inhibition of cell growth[a]%	
Dosage of *cis*-DDP (mg/kg)	Experiment 25-78	Experiment 31-78
8.00	98	
4.00	95	
2.00	93	
1.00	87	88
0.75		78
0.50		79
0.25		45

[a]Percent inhibition cell growth = (1 - mean No. cells in test group/mean No. cells in control group x 100).

Our goal was to determine a dose of each agent within the chosen dose ranges that would result in 25-45% tumor cell growth inhibition. These values are required for use in studies to detect possible synergistic antitumor effects of *cis*-DDP and CP. In the CP dose-response experiments, this dose level was not experimentally determined. A value of 20 mg/kg can be interpolated, however, from the data shown in Table 2. Our projected studies for the next quarter include combined use of CP and *cis*-DDP.

OTHER NUCLEAR MEDICINE TECHNOLOGY GROUP ACTIVITIES

Six shipments of ^{43}K were made to the University of Mississippi for coronary disease studies. Two shipments were made to the V. A. Center, Wood, Wisconsin for study of uptake in the hearts of stressed rats. One shipment was made to the National Institute for Environmental Health.

F. F. Knapp, Jr., and D. V. Woo attended the 19th Annual Meeting of the Southeastern Chapter of the Society of Nuclear Medicine at Birmingham, Alabama, on November 1-3. On December 7-8, F. F. Knapp, Jr., attended the Department of Energy Nuclear Medicine Contractors Meeting at Albuquerque, New Mexico.

Visitors for this period included Professor A. Tobias from the Chemistry Department at Purdue University who visited the Nuclear Medicine Technology Group on November 18 to discuss general aspects of the nuclear medicine research program. On November 19, Dr. P. Hyde

from the Biochemistry Department at Louisiana State University Medical School visited to discuss the potential use of ^{123m}Te-labeled steroids for adrenal imaging. Extensive discussions were held with Dr. P. J. Douglas from the Amersham Corporation on November 30 concerning the development of tissue specific ^{123m}Te-labeled agents. Dr. A. H. W. Nias from the St. Thomas Medical School, London, England, visited on November 13-14 to discuss establishing a Medical Cooperative Program to investigate the pharmacokinetic properties of novel ^{195m}Pt-labeled antitumor agents. In addition, a Radiation Biology Class from the University of Tennessee vistied the facilities on November 8. On November 7 a group of physicians and technicians attending an ORAU "Medical Planning and Care in Radiation Accidents" class visited the facilities, and on December 8, a group of students from a "Radiation: Its Production, Uses, and Dangers" class at Hiram College in Ohio toured the area.

PAPERS AND PUBLICATIONS

Papers

F. F. Knapp, Jr., K. R. Ambrose, A. P. Callahan, R. A. Grigsby, and K. J. Irgolic, "Te-123m-Labeled Isosteres of Unsaturated Fatty Acids-A New Class of Potential Myocardial Imaging Agents", 19th Annual Meeting, Southeastern Chapter, Society of Nuclear Medicine, Birmingham, Alabama, November 1-4, 1978.

D. V. Woo, K. R. Ambrose, A. P. Callahan, and F. F. Knapp, Jr., "Radiation Dosimetry of Te-123m-Labeled 3β-Hydroxy-24-Nor-23-(Isopropyl Telluro)-5α-Cholane-A Potential Adrenal Imaging Agent", 19th Annual Meeting, Southeastern Chapter, Society of Nuclear Medicine, Birmingham, Alabama, November 1-4, 1978.

Publications

N. P. Johnson, J. D. Hoeschele, N. B. Kuemmerle, W. E. Masker and R. O. Rahn, "Effects of Platinum Antitumor Agents and Pyrimidine Dimers on the *In Vitro* Replication of T7 DNA", Chem.-Biol. Interactions, 23, 267 (1978)

D. V. Woo, A. F. Rupp, and J. K. Poggenburg, "Microscale Synthesis of Phosphorus Trichloride Labeled with High Specific Activity ^{33}P", *J. Labeled Compd. Radiopharm.* XV, 117 (1978)

Reports

F. F. Knapp, Jr., *Nuclear Medicine Progress Report for Quarter Ending September 30, 1978*, ORNL/TM-6639.