COMPARISON OF TUMOR LOCALIZING PROPERTIES OF COBALT-57 BLEOMYCIN AND FOUR ANALOGUES: BLEOMYCINIC ACID, PHLEOMYCIN, PEPLEOMYCIN AND TALLYSOMYCIN

by

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STATEMENT BY AUTHOR

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ABSTRACT

The purpose of this study was to compare bleomycin and other bleomycin-type antibiotics as radiolabeled tumor-seeking agents. Bleomycin and 4 analogues labeled with Co-57 were examined for biological distribution in an established bleomycin-sensitive tumor model, the Ridgway osteogenic sarcoma, transferred by subcutaneous passage in AKD2-F1 mice. Radiolabeling was checked by thin-layer chromatography using methanol:10% ammonium acetate solution (1:1) as the solvent. Biological distribution studies were performed at 4 and 24 hours after intravenous administration. The following table shows the tumor-to-muscle and tumor-to-blood ratios obtained (average of 5 or more animals):

	4 Hours		24 Hours	
	T/M	T/B	T/M	T/B
Bleomycin	24.6	49.2	52.5	61.0
Pepleomycin	21.4	23.0	86.2	102.0
Bleomycinic Acid	17.9	108.3	41.9	57.8
Tallysomycin	8.8	14.7	25.1	15.9
Phleomycin	13.0	18.2	15.7	47.0

Absolute tumor uptake of the tracer was highest for pepleomycin, followed in descending order by tallysomycin, bleomycin, phleomycin, and bleomycinic acid. Pepleomycin, and possibly also bleomycinic acid, may be superior to bleomycin for tumor localization on the basis of high tumor-to-non-tumor ratios.

CHAPTER 1

INTRODUCTION

Studies on the localization of dyes and foreign proteins in spontaneous and transplanted tumors were initated in 1939 by Duran-Reynals. The differences in concentration of these substances found in animal and tumor tissues made it possible to visualize tumors by external detection of gamma-emitting radionuclides. Although instrumentation has steadily improved, the lack of radiolabeled compounds which specifically localize in tumors has remained the limiting factor in this field. Different histological types of tumors contain markedly different concentrations of the same radiolabeled substance. Locksley et al. (1954) have shown that studies in tumor-bearing mice help to predict the relative usefulness of different compounds. They found less variable results with a single transplantable tumor type in a pure strain of mice than in studies of patients.

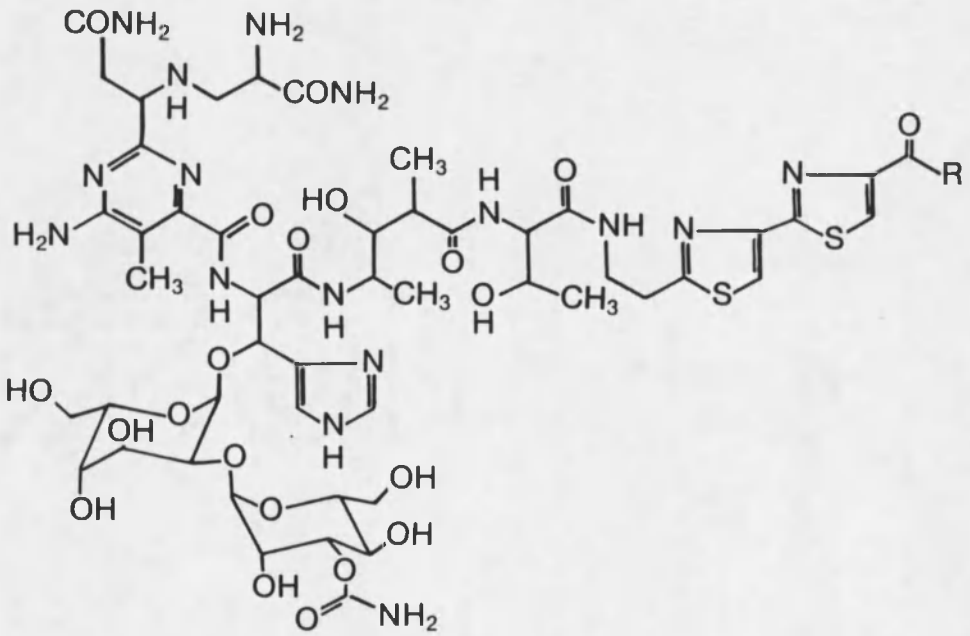
The characteristics of an "ideal" radiolabeled compound for external localization of tumors using a scintillation camera in nuclear medicine should include the following: 1) The substance must be labeled with a gamma-emitting radionuclide with suitable physical characteristics. 2) The tumor-to-normal tissue concentration ratio should be as large as possible. 3) The absolute concentration of radioactive material within the tumor bed should be high. 4) The blood-to-tumor concentration

ratio should be low. 5) The tumor-to-muscle concentration ratio should be high. 6) The concentration of radioactive material within the tumor must be maintained for a sufficient time to complete the scanning procedure. 7) The radioactivity should not become highly concentrated and retained in a particular organ or group of organs.

Bleomycin

Chemistry

Bleomycin is a water-soluble mixture of closely related antibiotics that has been used extensively in the treatment of various malignant tumors. Umezawa et al. (1966a) isolated this mixture of polypeptide antibiotics from a strain of streptomyces verticillus. It can be separated into at least 13 different bleomycin subtypes designated as A_1 to A_6, and B_1 to B_6, A'_2, with greater than 50% represented by the A_2 peptide. The preparation of fractions in the commercially available preparation has varied slightly from batch to batch without any apparent impact on the potency of the clinically used mixture. The fractions have an average molecular weight of 1400 and the structures of the main components (A_2; B_2) in the commercial preparation are shown in Figure 1. Although the bleomycin molecule is small by protein standards, it is quite complex and contains a variety of interesting amino acids and other functionalities. The structure elucidation was another accomplishment of Umezawa's group. The various bleomycins differ from one another with the "R" group being replaced by different terminal amines and can be separated by ion-exchange chromatography. Recently, Dell et al. (1980)



Bleomycinic Acid R = OH

Bleomycin A_2 R = NH $(CH_2)_3$ $S^+(CH_3)_2$

Bleomycin B_2 R = NH $(CH_2)_4$ NC(=NH_2^+)NH_2

Figure 1. Structural formula of bleomycin A_2, B_2 and bleomycinic acid.

confirmed the molecular weight of bleomycin B_2 by field desorption mass spectrometry

Toxicity

Acute Toxicity. The Physician's Desk Reference (PDR) (1978) reports that an anaphylactic reaction is seen in about 1% of the patients and is manifested by hyperpyrexia, shock, urticaria, and asthmatic wheezing. It is most frequently seen in patients with lymphomas that were treated with large single doses. There are also febrile reactions seen in about 25% of the patients. These are often severe and occur about 4-6 hours after administration. The incidence is greater with intravenous than with intramuscular dosing and usually become less frequent with use.

Chronic Toxicity. The most common side effects are the dermatological reactions that are seen in about 45% of the patients. Skin toxicity is a late manifestation, usually developing in the second and third week of treatment. It consists of erythema and inflammation of the hands, fingertip ulcerations, and hyperpigmentation over pressure areas. DeLena et al. (1972) report that pulmonary toxicity is the most serious adverse effect of this drug. It is reported to occur in about 10% of the treated patients. This reaction usually begins as dyspnea with fine rales, but in approximately 1% of the cases it may progress to fatal pulmonary fibrosis. Patients over 70 years old, especially those receiving more than 400 units or those with underlying lung disease, show a high incidence of pulmonary toxicity. Pasqual et al. (1973) suggest pulmonary function tests have not been particularly helpful in

predicting this complication. The manufacturer (Bristol Labs, Syracuse, New York) recommends taking a chest x-ray every one to two weeks. The abnormal chest x-ray shows bilateral basilar and perihilar reticulonodular infiltrates with fibrosis. These changes may occur up to a month after the drug is discontinued. If the vital capacity on a pulmonary function test decreases by more than 30-40% within four months, the drug must be discontinued. Lung biopsy shows interstitial edema, intraalveolar hyaline membrane formation, atypical alveolar epithelial cells, fibrinous edema, and in advanced stages, collagen deposition. Lehane, Hurd, and Lane (1975) report the drug is not clinically immunosuppressive. Sieber and Adamson (1975) suggest it is mutagenic and probably teratogenic. Edwards and Bernardino (1975) report it can also cause cataracts in rats. Fever, chills, and vomiting are frequently reported side effects. Anorexia and weight loss are common and may persist long after termination of this medicine. Pain at tumor site, phlebitis, and other local reactions have been reported.

Biochemistry

Carter et al. (1977) in a review report that bleomycin appears to exert its antitumor activity by directly binding to DNA, resulting in reduced synthesis of DNA, RNA, and proteins. Murakami, Mori, and Taira (1976) report it can also lead to single strand breaks in DNA through scission of thymine. The cytotoxic effects of bleomycin appear to be augmented by drugs acting by intercalation, radiation, and chemicals that generate superoxide radicals as reported by Ishida and Takahashi

(1975). Bleomycin should be considered cell cycle phase-nonspecific because cells in S, G_1, and G_2 are sensitive to bleomycin. Studies on the mechanism of action of bleomycin by Sausville, Peisach, and Horwitz (1976) have suggested that complex formation between bleomycin and Fe(II), followed by coordination with oxygen, results in generation of an oxygen radical anion which damages DNA. (Fe(II)-bleomycin + O_2 → Fe(III)-bleomycin + $^{-}O\text{-}O^{\circ}$). The Fe(III) can be reduced to Fe(II) in the presence of a number of reducing agents and thereby take part in many DNA-breaking events.

Metabolism

Bleomycin is enzymatically inactivated by many tissues. Umezawa (1974) has shown that bleomycins are subject to degradation by the action of an aminopeptidase found only in tumor cells, liver, and kidney. The degradative enzyme is notably absent from skin and lung--two parts of the body that are particularly vulnerable to the drug. Miyaki et al. (1975) have suggested, based on animal studies, that tumor cell resistance to bleomycin is also related to an increased ability to degrade the drug.

General Pharmacology

Bleomycin is active by all the usual parenteral routes of administration. In lower animals, it has a selective affinity for various tissues and is taken up poorly by hematapoietic tissues. The drug has a low therapeutic index and should be administered only to hospitalized cancer patients. Bleomycin sulfate occurs as a hygroscopic,

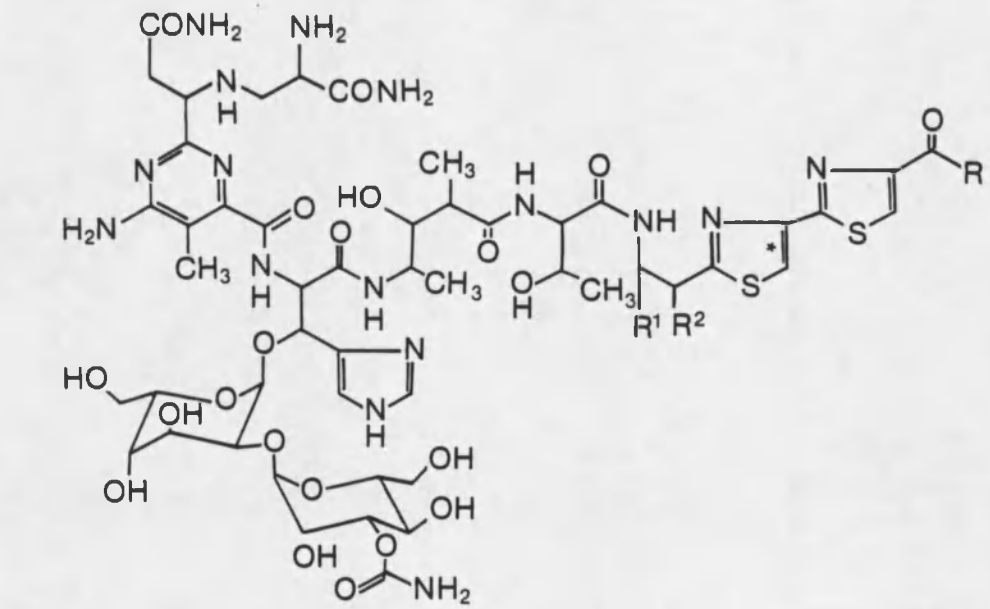
cream-colored powder. It is very soluble in water, giving solutions ranging from pH 4.5 to pH 6 that are stable for two weeks at room temperature. Sausville et al. (1976) have demonstrated that bleomycin is inactivated *in vitro* by sulfhydryl compounds, ascorbic acid, hydrogen peroxide, and heavy metal ions. Bleomycin is assayed microbiologically; 1 unit is equivalent in activity to 1 mg of bleomycin A_2.

Bleomycin pharmacokinetics have been studied by Ohnura et al. (1974) using a microbiologic assay. The microbiological method used in the studies was not sufficiently sensitive to provide a profile of drug disappearance for longer than 6 hours, and the assay was not able to distinguish between the several active bleomycin polypeptides or their metabolites. S. W. Hall et al. (1977) and Kramer et al. (1978) determined that after intravenous injection, bleomycin has a $T_{\frac{1}{2}}$ in plasma of two hours. It reaches high concentrations in the skin, lung, kidney, peritoneum, and lymphatics, but it appears to lack affinity for hematopoietic tissues. It is cleared primarily by the kidney, and clearance is markedly reduced in renal failure as demonstrated by Crooke et al. (1977). Bennett et al. (1977) have recommended that the dose of bleomycin be reduced as much as 50% in patients with severe renal failure. The regular dosage schedule is 10 - 20 units/meter^2 given IV, IM, or SQ, weekly or twice weekly. It has also been administered by the intra-arterial (Huntington, DuPriest, and Fletcher, 1973) and intracavitary routes (Cunningham et al., 1972), although such treatment has not been widely accepted. When bleomycin is used as a single agent, it produces partial remissions in a minority of patients for a short time. Yagoda et al. (1972) report responsive tumors include testicular neoplasms,

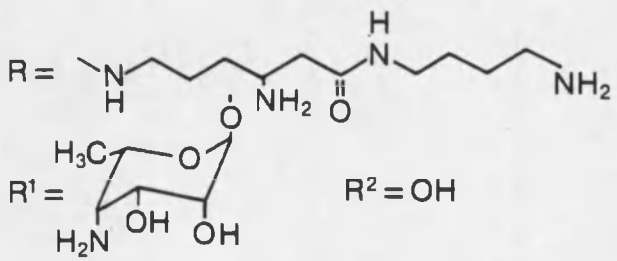
squamous cell carcinomas of the head and neck, ovarian adenocarcinomas, renal carcinomas, and soft tissue sarcomas. Activity also has been noted in Hodgkins disease and malignant lymphoma. The absence of bone marrow depression in most patients has led to the widespread use of bleomycin in a variety of drug combinations reported by Samuels et al. (1976) and DeVita, Serpick, and Carbone (1970). It has been used in conjunction with surgery and radiation therapy. However, the use of bleomycin in combination regimens as reported by Samuels et al. (1976), may intensify the skin and pulmonary toxicity of the drug, therefore, the treating physician must remain alert to toxic reactions.

Phleomycin

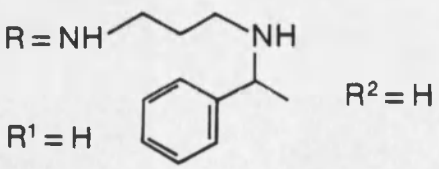
Phleomycin was discovered in 1956 prior to the development of bleomycin by Maeda et al. (1956). The structure of phleomycin shown in Figure 2 is very similar to bleomycin. The only differences are that phleomycin is partially reduced in one of its thiazole rings and has different terminal amines, as reported in a review by Remers (1979). Bradner and Pindell (1962) reported that this antibiotic was found to exhibit a high therapeutic index (TI) against Ehrlich carcinoma, sarcoma 180, and adenocarcinoma 755 in mice. Based on this high TI, a clinical study of phleomycin was designed for cancer patients. Remers (1979), in his chemistry review, reported that phleomycin caused severe renal toxity in dogs. As a result the planned clinical studies were withdrawn. The cumulative therapeutic dose of this antibiotic is toxic; however, the radiolabeled diagnostic dose would be only a tracer amount. Consequently,



Tallysomycin A



Pepleomycin



Phleomycin

R = several terminal amines
R¹ = H R² = H
* = no double bond

Figure 2. Structural formula of phleomycin, pepleomycin, and tallysomycin A.

the radiolabeling efficiency and biological distribution of this agent was evaluated.

Bleomycin analogs can be divided into three generations. First generation analogs are those discovered in the initial fermentation of bleomycin (i.e., bleomycinic acid). Second generation analogs comprise all those which contain the bleomycinic acid core, but differ in the N-terminal amine. They are made by fermentation precursors, chemical modification, or semisynthesis (i.e., pepleomycin). Third generation analogs are those which differ in the bleomycinic acid portion of the molecule (i.e., Tallysomycin). Matsuda et al. (1977) report that more than 300 artificial bleomycins have been produced and tested for anti-Hela cell activity and therapeutic indices against Ehrlich ascites carcinoma.

Tallysomycin

Recently, a member of the bleomycin group, Tallysomycin (Figure 2), was isolated by Kawaguchi et al. (1977) from an actinomycetes strain. Imanish et al. (1978) reported this agent showed antitumor activity in experimental animal tumor systems, including P-388 leukemia, sarcoma-180, Lewis lung, Walker carcinosarcoma-256, and melanoma B-16. Tallysomycin A is a modification of the bleomycinic acid core by the addition of an amino acid and a sugar moiety was reported by Strong and Crooke (1978). Bradner et al. (1977) demonstrated that it appears to have a therapeutic advantage over bleomycin in the Walker 256 carcinosarcoma in ascitic form and in P-388 leukemia in mice. Matsuda et al. (1978a)

demonstrated that Tallysomycin A is four or more times as toxic as bleomycin in both acute and chronic toxicity. Tallysomycin A, reported by Bradner (1978), caused a lower incidence of pneumonitis/fibrosis in chronically treated mice than bleomycin when the two drugs are given in equitoxic doses.

Bleomycinic Acid

The various bleomycins differ in the terminal amine moiety, with the bleomycinic acid core (Figure 1, where R=OH) present in all bleomycins. Therefore, a method for enzymatic hydrolysis of bleomycin A_2 and B_2 to bleomycinic acid and the terminal amine was reported by Umezawa et al. (1973) and Takita et al. (1973). Bleomycinic acid can be coupled with specific amines by use of a water soluble carbodiimide to form new semi-synthetic bleomycins.

Pepleomycin

Pepleomycin, a precursor-fed fermentation bleomycin (Figure 2), which contains N-(1-phenylethyl) diaminopropane as the terminal amine, was selected for a Phase I clinical study for the following reasons: a) higher antitumor activity, b) four times lower pulmonary toxicity, c) stronger effect on chemically induced squamous cell carcinoma and gastric cancer of experimental animals was demonstrated by Matsuda et al. (1978a, 1978b) and Takahashi et al. (1979). The clinical trial was carried out in 32 advanced or recurrent cancer patients. Favorable responses were seen in patients with the type of cancer sensitive to bleomycin. Furthermore, in cases not sensitive to bleomycin (prostatic

cancer and hepatoma), there were some responders. The incidence and degree of fever after injections were less and the patients tolerated pepleomycin well.

Radiolabeled Bleomycin Studies

Chemistry

The sites at which bleomycins chelate cations and why after bleomycin is chelated it greatly modifies the biological distribution is still under investigation. Bleomycin is isolated as the copper chelate, but the copper is removed with 8-hydroxyquinoline or EDTA prior to clinical use because the copper-free bleomycin has greater antineoplastic activity and less toxicity. Rao et al. (1980) studied the concentration-dependent cytotoxicity and antitumor activity of bleomycin (Blm) and Cu-Blm, Fe(III)-Blm, and Co-Blm using Ehrlich cells in culture and the Ehrlich ascites tumor. The order of activity in culture was Cu-Blm>Blm>Zn-Blm>Fe(III)-Blm>Co-Blm>control. The antitumor experiments produced qualitatively similar results with the order of potency being Cu-Blm>Blm>Zn-Blm>Fe(III)-Blm>Co-Blm>control tumor. The most toxicity measured by mouse weight loss had the opposite ordering: Co-Blm>Fe(III)-Blm>Zn-Blm>Blm>Cu-Blm. The studies of Umezawa (1977) have demonstrated that an intracellular mechanism exists for removing copper from bleomycin. Grove, Eckelman, and Reba (1973) showed that Fe(III) bleomycin is readily hydrolyzed in neutral aqueous solutions using thin-layer chromatography. Kono et al. (1977) demonstrated by thin-layer chromatography that Ga(III)-bleomycin is unstable in neutral and akaline solutions. Taylor and Cotrall (1975) found that tissue distribution of ^{62}Zn-chloride

was similar to ^{62}Zn-bleomycin and the complex dissociated in vitro. Orii and Oyamada (1974) found that ^{99m}Tc is associated with human serum albumin 4 hrs after injection of ^{99m}Tc-bleomycin so it is unlikely that this chelate remains intact in vitro. In spite of positive clinical results with ^{111}In-Bleomycin, within 4 hours after injection ^{111}In has been found bound to serum transferrin by Thakur, Merrick, and Gunaschera (1973). Kono et al. (1977) found that most of the ^{111}In in mouse urine was not bound to bleomycin. The most encouraging clinical results have been obtained with the ^{57}Co-bleomycin complex.

The structure as proposed by Sugiura (1979) is shown in Figure 3. However, Nunn (1977b) proposed that binding of Co(III) to the antibiotic yielded an air sensitive complex and a more stable Co(III) bleomycin chelate. By electrochemical studies, Dabrowiak and Santillo (1979) and Dabrowiak (1980) reported that 4-amino pyrimidine is bound to the cobalt ion. Titration studies by Suguira, Ishizu, and Miyoshi (1979) have confirmed that the primary and secondary amines and the imidazole functions are bound to Co(III). The fourth proton loss accompanying the binding of the drug to Co(II) is presumably due to a deprotonated amide function. Aerobic oxidation of Co(II) bleomycin ultimately results in formation of Co(III) bleomycin which DeReimer et al. (1979) reported to exist in two forms, an orange and a green complex; they can be separated using HPLC as reported by Vos, Western, and Van Zanten (1980).

High resolution (360 MHz) ^{1}H mmr spectroscopy deomonstrated chemical shifts for the imidazole and pyrimidine residues of the complex indicating metal chelation at these sites. Polarographic analysis by

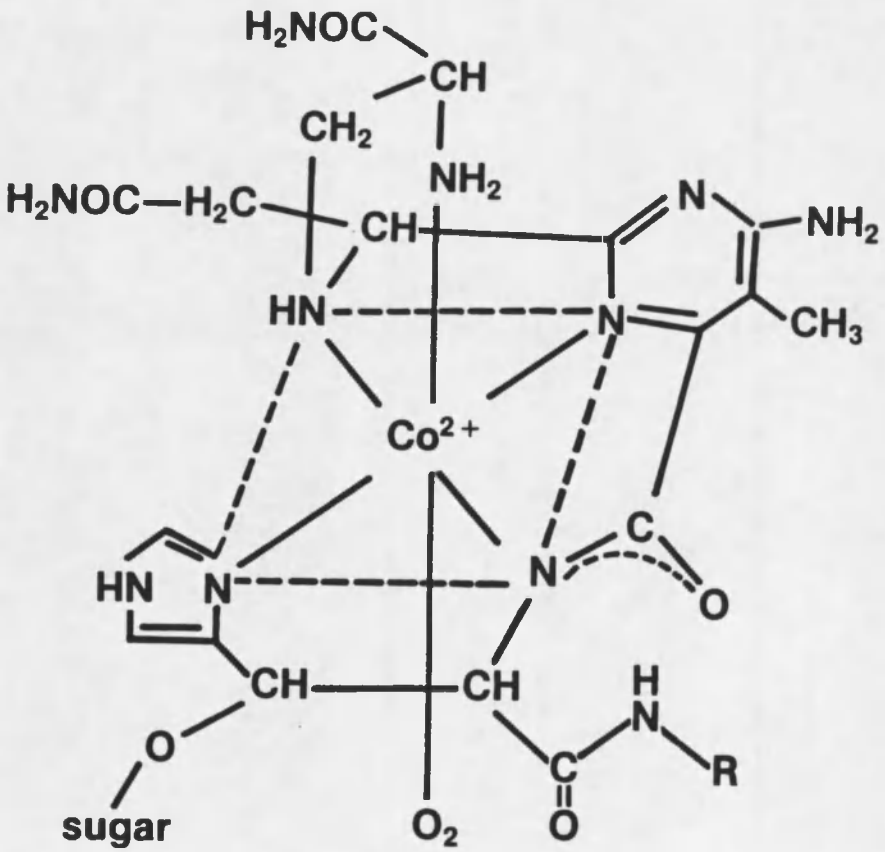


Figure 3. Structural formula of bleomycin-Co(II)-O_2 complex.

Dabrowiak and Santillo (1979) of Co(III) bleomycin has confirmed that the pyrimidine is a metal binding site. Vos, Western, and Schipper (1980), in a ^{13}C nmr and esr study for oxygenated cobalt-bleomycin A_2 proposed that all the cobalt is in the Co(III) state. The structure of the Co-bleomycin complex in which the nitrogens of pyrimidine, the imidazole, and the secondary and primary amine of the diaminopropanoic amide are involved as planar equatorial ligands. The free bleomycin is considered to form a "dimer" with Co-bleomycin, in which the bleomycin behaves as an axial ligand-cloud, which "hovers" above a Co-bleomycin complex. Nouel (1976) reported that ^{57}Co-bleomycin is characterized *in vitro* by rapid blood clearance (80% to 90% excreted in the urine within 24 hours) and a high affinity for the nuclei of tumor cells. Konings and Rasker (1978) reported that the highest radioactivity was in the subcellular fractions containing mitochondria and lysosomes. They concluded that this radiopharmaceutical is preferentially localized in the heavy secondary lysosomes of the tumor. The distinguishing feature of the cobalt-bleomycin chelate as opposed to the other cation-bleomycin chelates is the stability of the complex. Kono et al. (1977) reported that incubating ^{57}Co-bleomycin with a threefold excess of EDTA for 30 days resulted in no transfer of cobalt. Incubation of the cobalt bleomycin complex by Nunn (1977a) for 18 hours with a ten-fold excess of CO(II) or Cu(II) caused less than 5% of cobalt to be displaced. Consequently, of all the metal ion-bleomycin complexes investigated to date, only that of ^{57}Co has been found to remain intact *in vitro*.

Biological Distribution in Animal Tumor Models

Much attention was drawn to a new method of visualizing tumors after the promising results of Nouel et al. (1971) with the gamma-emitting Co-57 as a radiolabel for an antineoplastic agent (bleomycin). Since bleomycin chelates divalent and trivalent cations, Ryo et al. (1975) and Van de Poll et al. (1976) published their experiences with other radiolabels (^{67}Cu, ^{67}Ga, ^{111}In, ^{62}Zn, ^{99m}Tc, ^{197}Hg, ^{203}Pb) in experimental tumors in animals. In addition, a covalently radiolabeled bleomycin (^{131}I) was reported by Meyers, Krohn, and DeNardo (1975).

Merrick et al. (1972) reported encouraging initial results in 17 patients with ^{111}In-bleomycin for the scintigraphic diagnosis of tumors. ^{111}In-bleomycin was found to concentrate in tumors more effectively than ^{99m}Tc or ^{67}Ga bleomycin. Consequently, the first radiolabeled bleomycin available commercially under an IND from the Medi-Physics Corp., Emeryville, California was ^{111}In-bleomycin. Lilien et al. (1975) have evaluated ^{111}In-bleomycin in 293 patients. Among 246 of these with cancer, 218 (89%) were true positive and 28 (11%) were false negative. Advantages of the ^{111}In-bleomycin studies reported by Poulose et al. (1975) were that a broader spectrum of tumors were positive and there was better delineation of abdominal and pelvic disease due to lack of normal gut uptake than with ^{67}Ga-citrate. Grove et al. (1973) found a higher tumor/blood ratio with Co-57 bleomycin than with bleomycin radiolabeled with ^{111}In and ^{67}Ga. Eckelman et al. (1975a) demonstrated that ^{125}I-bleomycin was distributed simultaneously to unlabeled bleomycin, but concentrated less in animal tumors than ^{57}Co-bleomycin. Kono et al.

(1977) also demonstrated that the tissue affinity and intercellular distribution of ^{57}Co-bleomycin differs from bleomycin and that the chelate is less active as an antineoplastic agent. DeNardo et al. (1975) found that ^{131}I-bleomycin concentrated in tumors more effectively than ^{67}Ga or ^{111}In-bleomycin. In most of the studies that compared radiolabeled bleomycin to other radiolabeled compounds using the same radionuclides, the former portrays higher affinity for the tumor. Merrick et al. (1975), in a comparison of various ^{111}In-compounds with ^{111}In-bleomycin, reported no significant difference in tumor affinity. Robbins, Silverstein, and Fortman (1974) found that ^{111}In-bleomycin was cleared more slowly from the blood than ^{57}Co-bleomycin, and that the tumor to blood ratios of ^{111}In-chloride and ^{111}In-bleomycin were similar. Thus, they suggested in vivo dissociation of ^{111}In-bleomycin lavel. Thakur et al. (1973) have shown that the ^{111}In of ^{111}In-bleomycin is found on transferrin with 4 hr post-injection in man, which suggested competition by transferrin for the ^{111}In radiolabel and/or exchange of serum cations for the chelated Indium-111. Hall, O'Mara, and Cruz (1974) reported that ^{67}Cu-bleomycin is superior to ^{111}In-bleomycin in visualizing tumors in animals, while Eckelman et al. (1974) found ^{64}Cu-bleomycin inferior to ^{57}Co-bleomycin in visualizing tumors in rats. Investigations of the radiolabeled bleomycins have been hampered by the lack of uniformity of using the appropriate experimental animal tumor model. For example, a limitation of the solid transplantable tumors commonly used in chemotherapeutic research is that their growth is arrested by the antineoplastic agent only if treatment is started before the tumors are well established in the host. All of this confusion prompted our group (Hall et al., 1976)

to perform a comparative study of radiolabeled bleomycin and the ionic radioactive species in a proven bleomycin-sensitive animal tumor model. On the basis of in vitro and in vivo results, Co-57 bleomycin reported by Raban, Brousil, and Svihovcova (1979) demonstrated superior tumor localizing properties over all radiolabels studied. Since bleomycin is a mixture of basic cytotoxic glycopeptide antibiotics, Eckelman et al. (1975b) isolated the various fractions from the commercial preparation by HPLC and investigated the chemical and biological properties of the ^{57}Co labeled fractions. They found that fractions A2 and B2 achieve high tumor-to-non-tumor ratios in a Fischer-344 rat bearing a 13762 mammary adencarcinoma. J. N. Hall et al. (1977) evaluated the artificial bleomycins produced by fermentation rather than HPLC to eliminate the potential for undesirable chemical reactions (e.g., transformation of A2 by solvent system used in separation to DMA_2). They found that Co-57 bleomycin A2 gave the highest tumor-to-non-tumor ratios. Kakinuma, Kagiyama, and Orii (1980) reported the biological distribution of Cm-Sephadex C-25 column separated radiolabeled isomers of bleomycin. They showed higher tumor-to-blood and tumor-to-muscle ratios when compared to the radiolabeled bleomycin commercial mixture. However, the differences between the four isomers were not significant. They postulated that the groups on the bleomycin molecule which are involved in chelate formation, do not take part in its binding to DNA. The unsatisfactory tumor localization of radioiodinated bleomycin and of bleomycin labeled with other cation radionuclides suggests that cobalt-labeled bleomycin has unique properties, most likely relating to the chelated cobalt, which are

important in determining the high tumor-to-non target ratios achieved with this radiopharmaceutical.

Clinical Studies with ^{57}Co-Bleomycin

The report by Nouel et al. (1971) was the first in the medical literature on use of bleomycin labeled with ^{57}Co. Ten patients with carcinoma involving different sites were evaluated by scintillation scanning. They detected unsuspected metastasis in 4 cases, and found an occult primary tumor in 2 patients with known metastasis. Toti and Ciaccia (1973), in Italy, reported results of ^{57}Co-bleomycin scans in 101 patients. Scans were positive in 62 of the 65 patients with neoplasm and 4 of the patients with benign conditions (silicosis-2, sarcoidosis-1, pulmonary fibrosis-1). Laconi, Gallo, and Torina (1973), also from Italy, summarized results of 11 patients with 10 of the 11 showing uptake at the neoplastic sites. There was one false negative in a patient with previously irradiated hilar and mediastinal metastases from a seminoma. Suzuki et al. (1974), in a series of 46 patients, demonstrated positive scans in 24 of 36 patients with malignancies. Nouel et al. (1974), presented the largest series of Co-57 bleomycin diagnostic studies (1000 patients). Out of 54 patients with metastatic neoplasms of unknown source, a primary tumor was discovered in 17 using the Co-57 bleomycin technique. Truhant et al. (1975) reported ^{57}Co-bleomycin scanning in 92 patients with known malignancies; a total of 126 neoplastic sites were evaluated. One hundred sixteen sites were positive, and 10 were negative on the scans. Lucat et al. (1976), in a series of 97 patients with intrathoracic abnormalities, 44 of 47 patients with

pulmonary tumors portrayed ^{57}Co-bleomycin uptake in the lesions. Eight of 17 non-cancerous lesions showed uptake at sites of inflammation or surgical incisions. Rasker et al. (1975), from the Netherlands, evaluated ^{57}Co-bleomycin scanning of hilar and mediastium in 50 patients with bronchial carcinoma. Sensitivity of ^{57}Co-bleomycin scanning was 100% for hilar lesions, 80% for mediastinal lesions, and 92% for hilar and mediastinum taken together. Kahn et al. (1977) reported results in 140 patients with Co-57 bleomycin imaging with 82 of 115 patients with active disease demonstrating positive scans. Reba et al. (1974) reported that ^{57}Co-bleomycin was found to be slightly superior to Ga-67 citrate in detecting tumors other than lymphomas. Mamo et al. (1973) demonstrated that ^{57}Co-bleomycin was found to be superior to ^{99m}Tc-bleomycin in detecting intracranial tumors. Silberstein (1976), in a review, portrayed Co-57 bleomycin scanning to be a more sensitive test than scanning with bleomycin labeled with other radionuclides.

Woolfenden et al. (1979) reported utilizing ^{57}Co-bleomycin as an agent for imaging the extent of head and neck tumors. The results indicate that this agent should be useful for this purpose because the tumor to background ratio is much higher than with ^{67}Ga citrate. A recent study of a larger patient population by Cummings et al. (1981) reported that individuals with tumors greater than 2 centimeters in size with head and neck cancer appear to demonstrate active uptake of the imaging agent. False positive results (10%) were seen in inflammatory conditions or benign tumors of the salivary glands.

After the promising clinical results with Co-57 bleomycin obtained by several investigators, it appears that analogues of bleomycin should first be evaluated with Co-57 as the radiolabel. The purpose of this study is to compare the biological distribution of bleomycin and four analogues labeled with Co-57 in an established bleomycin-sensitive animal tumor model.

CHAPTER 2

EQUIPMENT AND SUPPLIES

Equipment List

Gamma ray scintillation detector:

For counting dose syringes:

NaI(Tl) crystal (well type)

Raytheon

Model 210

High Voltage: 658v

Setting: 100-200keV

Syringe holder: First level

Range: 100-1100

For counting tissue samples:

NaI(Tl) crystal (well type)

Searle Analytic

UA# A114332

Attenuator: coarse: 8 fine: 20

Differential: wide

Base: 100

Window: 100

High Voltage: 1050v

Gamma-camera:

Searle Radiographics Pho/Gamma-IV, Des Plaines, IL.

Thin-layer chromatographic analysis:

Automatic:

Searle: Actigraph

UA# A135001

High Voltage: 952v

Seconds: 0.5

(lead plate attached for gamma ray attenuation)

Multichannel Analyzer:

UA# A132207

Tracor-Northern TN 1710

Preset livetime

Conversion Gain: 1024

Anesthesia:

Ether

Sodium Pentobarbital

Nembutal^{R} Sodium injection

Abbott Labs

50 mg/ml

Optimum dose: 0.06 mg/g

Radionuclide:

1) Cobalt-57 in 0.5 N HCl (New England Nuclear, North Billerica, MA 01862) or ^{57}Co in 0.1 N HCl (Amersham/Searle Corp., Chicago, IL) is obtained under Arizona AEC Board License #10-44.

2) In-111 Chloride in 0.1 N HCl (Medi-Physics Corp., Emeryville, CA).

3) Cu-67 chloride in 0.1 N HCl (Oak Ridge National Lab., Oak Ridge, Tenn.).

Pharmaceutical:

Bleomycin

Blenoxane^{R}

Bleomycin injection - 15 units/ampul (Bristol Laboratories, Syracuse, NY 13201).

Betadine^{R}; povidone-iodine (Purdue Frederick Co., Yonkers, NY)

Xylocaine^{R}; lidocaine (Astra Pharmaceutical Products, Inc., Worcester, MA), 1% solution

Sparine^{R}; promazine HCl (Wyeth Labs, Philadelphia, PA), 50 mg/ml

Ketaject^{R}; ketamine HCl (Bristol Labs, Syracuse, NY), 100 mg/ml

Lipo-Hepin^{R}; heparin sodium sterile solution - 1000 u/ml (Upjohn Co., Kalamazoo, MI)

Thin layer chromatography studies:

Polygram^{R} Sil G (Brinkman Instruments, Inc., Westbury, NY)

Layer: 0.25 mm silica gel without gypsum

Batch 17

Solvent:

1:1 Methanol:10% Ammonium Acetate solution

Various needles and syringes:

Monoject

HR1 # = 8881-501210

Size = 1cc

Graduations = 100 units

Needle size = 27 ga x ½"

Sherwood Medical, St. Louis, Mo. 63103

Millipore Filter - 0.22 μm

Millipore Corp., Bedford, MA 01730

Cell medium:

McCoy's Medium 5a

Catalog # 320-6610

Control # C183310

Grand Island Biological Company

Grand Island, New York

Reaction vial:

A 10 ml sterile pyrogen-free serum vial (Elkins-Sinn, Inc., Cherry Hill, NJ 08002).

Sodium chloride 0.9% injection USP:

Travenol Laboratories, Inc., Deerfield, IL 60064

Sodium acetate injection:

2 mEq/ml (Abbott Laboratories, North Chicago, IL 60064).

Laminar Flow Hood (Pure Aire Corporation, Van Nuys, CA)

Gelman UV Lamp (Gelman Corp., Ann Arbor, MI)

Sterile Tissue Culture Dish (Falcon #3007, Oxnard, CA)

Tissue Sieve - 40 mesh (E-C Apparatus Corp., St. Petersburg, FL)

Micro-test tubes (Bio-Rad Laboratories, Richmond, CA)

Brinkman Microcentrifuge Model #1101 (Brinkman Instruments, Westbury, NY)

Mettler Model P-163 Analytical Balance (Mettler Instrument Corp., Princeton, NJ)

Plastic test tubes (Falcon #2025, Oxnard, CA)

4-0 Suture Material (Deknatel, Inc., Queen's Village, NY)

Silastic Tubing - 0.047" O.D. x 0.025" I.D. (Dow Corning, Alhambra, CA)

Mouse and Rabbit Cages (Hoeltge Co., Cincinnati, OH)

VAX-11/780 Computer (Digital Equipment Corporation, Maynard, MA)

Cyber-175 Computer (Control Data Corporation, Minneapolis, MN)

CHAPTER 3

EXPERIMENTAL PROCEDURES

Experimental Method

The mouse tumor used in the quantitative biological distribution study was the Ridgway osteogenic sarcoma (ROS) subcutaneously implanted by serial passages at 21 day intervals into an AKD_2F_2 hybrid, a first generation cross between an AKR female and a DBA/2 male. This hybrid is a much more vigorous animal than the inbred line of origin, the AK mouse. The ROS tumor was first observed in November 1948 by Dr. J. H. Burchenal at the Sloan-Kettering Institute (SKI), New York, N.Y., as a spontaneously appearing inguinal mass in a male AKM mouse. He gave the tumor-bearing mouse to Dr. D. A. Karnofsky, who established the transplantable tumor in his laboratory. Dr. Karnofsky names it "Ridgway" for Lois Ridgway, the technician in his laboratory who carried out this early work. This tumor was obtained in July 1975 from Linda Simpson-Herren, Southern Research Institute, Birmingham, Alabama. The tumor subline was ROSI-$a_1b_1c_1$-26 which was implanted 7-21-75 and at 14 days post implant was about 0.5 grams. Histologically, the original spontaneous tumor was made up of solidly packed round cells with foci of bone formation. Sugiura (1979) reported that after a few subcutaneous passages in AKM mice, it lost bone structure but retained high alkaline phosphatase activity. Laster (1975) reported that the "established" tumor is sensitive to the best known and most widely used DNA binders and intercalators (e.g., Bleomycin) and that

the AKD_2F_2 hybrid was a more vigorous animal than the AKR mouse. Goldin and Kline (1978) reported a marked increase in life span (67% ILS) with daily treatment with bleomycin on days 2 through 11.

The scintillation camera imaging studies were carried out in New Zealand white rabbits bearing a V-2 carcinoma in the right hind leg. The tumor was transferred by intramuscular injection in saline after obtaining a cryo-preserved suspension from Dr. Arthur Bogden (Mason Research Institute, Worchester, Massachusetts) in September 1976. This tumor was derived from a transformed papilloma induced by the Shope virus and its origin reported by Drs. Kidd and Rouse in 1937, was submitted to Mason Research Institute by Dr. P. Gullins. On histological examination, the tumor has an "anaplastic vascular carcinoma" type pattern.

Sensitivity of the V-2 carcinoma to bleomycin has not been reported, but high tumor to background ratios were demonstrated by Ito et al. (1975) and excellent visualization of this tumor was portrayed by Meares, DeRiemer, and Goodwin (1978).

Drugs

Bleomycin, as a mixture of glycopeptides (Lot #H9X08), was obtained from Bristol Labs, Syracuse, N.Y. The bleomycinic acid (Lot #H-7286) was a generous gift from Dr. Wataru Tanaka, Nippon Kayaku Inc., Tokyo, Japan. The phleomycin, tallysmycin A, and pepleomycin were obtained through the courtesy of Dr. John Douros, Natural Products Branch, National Cancer Institute, Bethesda, Maryland.

Preparation of Cobalt-Bleomycin and Analogues

Cobalt-57 chloride is produced by New England Nuclear using a ^{58}Ni (p,2n) ^{57}Co reaction, and is sold as a radiochemical in 0.5 N HCl with a specific activity of 5 Ci/mg. In order to prepare the bleomycin complex, the ^{57}Co in 0.5 N HCl is diluted to 0.1 N HCl with sodium chloride 0.9% injection, and is aseptically transferred with a hypodermic syringe and needle to a 10 ml serum vial. In a typical batch, 0.2 - 1.0 mCi of radioactivity are employed. Bleomycin (Bristol Laboratories) or the analogue is reconstituted fresh in sodium chloride 0.9% injection, and added to the serum vial in a quantity sufficient to obtain a concentration of 1 mCi ^{57}Co per 1 unit of bleomycin or 5 mg analogue. The pH of the solution is adjusted to approximately pH-6 with sodium acetate for injection (2mEq/ml), and the resultant pH is checked with pH paper. The complex is then diluted with sodium chloride 0.9% injection, so that the final ^{57}Co concentration is 200 μCi per ml. The entire contents of the reaction vial are then removed into a new syringe and filtered with a 0.22 μm millipore filter into a new, appropriately labeled sterile-pyrogen free 10 ml serum vial which is the final drug container. The final solution is checked for clarity, absence of color, and absence of particulate material. All compounding steps are carried out in a clean laminar flow hood (Pure Aire Corporation, Van Nuys, CA 91406).

Radiochemical and Radionuclide Purity

The radiochemical purity and labeling yield were determined by the following procedure: Place a drop of the Co-57 radiolabeled compound

approximately 25 mm from one end of a 25x200 mm silica gel TLC strip (Brinkman Instruments) and allow to dry. Develop the chromatogram over a period of 3 hours by ascending chromatography using methanol:10% ammonium acetate solution (1:1) as the solvent and dry it in air. Observe the strip under a Gelman uv lamp (254 nm) for fluorescence and circle the fluorescent areas. The radioactivity distribution was determined by scanning the chromatogram with a Searle Actigraph III radiochromatographic scanner. In addition, for quantitation, strips were cut into 5 mm widths and counted in a Model #1105 Searle Analytic NaI (TI) automatic gamma counting system. Radionuclide purity of the Co-57 chloride was checked with a Model #1705 Tracor Northern multichannel analyzer coupled with a NaI (TI) scintillation detector (less than 0.3% Co-56 and 0.1% Co-58 is acceptable).

Preparation of Tumor Suspension

All equipment is sterile and cleanliness is observed. Sacrifice the donor tumor animal with ether and the entire tumor area is swabbed with 70% isopropyl alcohol. The skin is incised circumferentially (with scissors) around the tumor, away from the tumor mass, and is reflected over a finger. The tumor is removed using scalpel and forceps to a sterile tissue culture dish (Falcon #3007, Oxnard, CA) and minced with scissors in an appropriate media (McCoys 5a with 5% fetal calf serum - mouse; saline - rabbit). A single cell suspension is formed by forcing the tumor through a 40 mesh tissue sieve (E-C Apparatus Corp., St. Petersburg, FL). An aliquot is stained with Trypan blue dye and the number of viable cells are counted under the microscope in a haemocytometer.

Dilute the suspension with an appropriate media to a concentration of $4x10^6$ viable cells/ml for either tumor transplantation or for *in vitro* radioactivity uptake experiments.

Tumor Transplantation Technique

Morphologically and physiologically there are great varieties of transplanted tumors distributed among many inbred strains of animals. Most tumors are maintained as solid subcutaneous growths in animals of the same strain. Solid tumors show wide individual variations in their growth rates. Male AKD_2F_2 mice (the Jackson Laboratory, Bar Harbor, Maine) 6-8 weeks old and weighing 25-35 g were housed 5 to a cage. The New Zealand white rabbits (Blue Ribbon Rabbitry, Tucson, Arizona) weighing at least 1.5 kg were housed one per cage.

Animals are maintained under standard laboratory conditions: 22±2°C, 55±5% humidity, air exchange 20 times per hour. The animals receive a normal diet and water ad lib. Hosts are clipped free of hair over the inoculation site (over the rump) and the skin is swabbed with 70% isopropyl alcohol. The mouse tumor cells are dispersed by pulling them into and out of a syringe (1 ml) with an 18 gauge needle. Then the cells are taken up into the syringe and stood with the needle tip up to allow cells to settle in the syringe. The excess fluid is ejected to a 0.2 ml volume. The host mouse is anesthetized (ether) and injected with the 0.2 ml tumor suspension. Tumor growth is usually evident within a week of transplant, but, in any event, newly inoculated animals should be checked at not less than weekly intervals. Solid tumors should be transplanted before ulceration appears in the overlying skin. The rabbit

tumor is transplanted using a 13-gauge Trocar because the V-2 carcinoma is a naturally hard tumor and difficult to mince. Briefly, the trocar is a spinal tap needle of which the plunger tip has been rounded off and the barrel ground to a sharp point similar to that of a hypodermic needle. A small amount of tumor is loaded into the 13-gauge trocar; the trocar is pushed through the skin in the shaved area and the tumor expelled. Tumors (0.5 - 2 cm in diameter) were present in 70 percent of the injected animals 3-4 weeks later, and radionuclide uptake studies were conducted at that time.

In Vitro Uptake of Radioactivity by ROS Tumor Cells

In order to determine if bleomycin facilitated uptake of the radiolabel into the ROS tumor cells an *in vitro* experiment was performed. The uptake of radioactivity by the cells was determined for ^{111}In-Chloride, ^{111}In-bleomycin, ^{67}Cu-Chloride, ^{67}Cu-bleomycin, ^{57}Co-Chloride, and ^{57}Co-bleomycin at varying time intervals in micro-test tubes (Bio-Rad Laboratories, Richmond, CA). The test tubes are incubated at 37°C with the various radiolabels (0.5 μCi; 0.0002 u bleomycin) by suspending $4x10^6$ viable cells in 1 ml of McCoy's 5a media with 5% fetal calf serum (Grand Island Biological Co., Grand Island, NY). The total radioactivity was measured in a Searle Analytic Model 1105 sodium iodide (Tl) gamma scintillation well counter during the incubation period. The cells are then centrifuged at 800-100 x gravity in a Brinkman Model #1101 micro-centrifuge (Brinkman Instrument Co., Westbury, NY). The supernatant was decanted and the cells were washed one with McCoy's 5a media and

recounted in the gamma counter. The recorded impulses are corrected for background and are expressed as counts per minute x 10^4 per $4x10^6$ viable ROS cells.

Biological Distribution Studies in Mice

Groups of five ADK_2F, male mice (20-30 grams) are injected intravenously (tail vein) with 0.2 ml (approximately 20 μCi) under pentobarbital anesthesia. Intravenous injection is the preferred procedure because bleomycin is not active orally and it avoids the complication of an absorption phase that is seen with other routes of administration. This permits a more precise calculation of the initial rates of compound uptake and clearance. The total body weight of each animal was recorded, and the quantity of radioactive material injected was carefully measured by counting the syringe for each animal before and after injection. These counts were obtained by using a personally designed distance bar apparatus for a Raytheon Model 210 sodium iodide (Tl) scintillation well counter (Raytheon Company, Waltham, MA). One of the syringes containing a dose of each radioactive compound was injected into a water filled 100-ml volumetric flask containing a small amount of non-radioactive bleomycin and cobaltous chloride (carrier to prevent glassware binding of radioactive tracer). This flask was diluted to 100 ml with water for use as a counting standard containing a known fraction of the administered dose.

The mice are sacrificed with ether at 4 and 24 hours following injection. Immediately following sacrifice a heparinized blood sample was obtained by intracardiac puncture. Samples (0.5 grams) of organs of

interest are excised and weighed (wet weight) on a Mettler model P-163 analytical balance (Mettler Instrument Corp., Princeton, NJ). The aliquots of blood as well as organs are placed in individual plastic capped test tubes (Falcon #2025, Oxnard, CA) and the radioactivity is measured in a Searle Analytic Model 1105 automatic sodium iodide thallium activated gamma well counter. The percent of administered dose per gram of organ was determined by comparison of tissue counts to the diluted aliquots prepared at the time of administration to the mice.

Radioactivity in the tails was counted and any mouse with more than 10% of the injected dose in the tail is excluded from the study. The tissue weights and counts per minute, and the counts per minute of the dose and standard syringes are entered into a computer program, GAMCNT (Appendix A), to calculate absolute tissue uptake (% dose/g) and the tumor-to-organ ratios. The tumor-to-blood and tumor-to-muscle ratios are then entered in groups into a computer program, ANOVA2 (Appendix B), which calculates the F-ratios for each of the following comparisons: differences between compounds at a given time, between times for a given compound and between compounds at different times. For those groups that showed a significant difference ($p < 0.01$) in the analysis of variance test, a Student's "t" test was carried out ($p < 0.05$).

Scintigraphy

Imaging studies were performed on anesthetized tumor-bearing rabbits using a gamma camera with whole body imaging table (Searle Radiographics Pho/Gamma IV) at 24 hours after intravenous injection of

200 μCi of the Co-57 bleomycin analogue. The camera was adjusted to the 122 keV photopeak, with a 20% window, and the high sensitivity collimator was employed. About 30-45 minutes were required per view so the rabbits were anesthetized by intramuscular injection of 25 mg of promazine HCl (Wyeth Labs, Philadelphia, PA), 200 mg of ketamine Hcl (Bristol Labs, Syracuse, NY). Whole body images of a rabbit with bleomycin acid was not obtained due to small sample size received from Dr. Tanaka.

Procedure for Pharmacokinetic Studies

The usual procedures of taking multiple blood samples from the marginal ear vein of rabbits proved inadequate in our laboratory. The procedure used is similar to that developed by Popovic and Popovic (1960) for rats and ground squirrels and modified by Hall et al. (1974) for rabbits. Briefly, the technique involves inserting a catheter into the right external jugular vein and down into the right anterior venacava of the rabbits. The rabbits are injected with 25 mg of promazine HCl 15 minutes prior to injection of 200 mg of ketamine Hcl. The neck is shaved with animal clippers and painted with povidone-iodine (Betadine-Purdue Frederick Co., Yonkers, NY). The incision site is infiltrated with 1% lidocaine (Xylocaine-Astra Pharmaceutical Products, Inc., Worcester, MA) to produce a local anesthetic effect. A 4-6 cm medial ventral skin incision is made from the level of the hyoid bone and extending toward the sternum. The muscle and fat are separated from the external jugular vein with a small curved hemostat. A 4-0 suture

(Deknatel, Inc., Queen's Village, NY) is placed under the vein on each side of a point where a small incision will be made in the vein. The free ends of each strand are clamped (about 2 cm apart) with hemostats and the heparinized silastic tubing (0.047" O.D. x 0.025" I.D.-Dow Corning, Alhambra, CA) is maneuvered slowly so that the tip lies approximately within the right anterior venacave (10-12 inches). Withdrawal of a blood sample ensures that the catheter is inserted properly and then refilling by irrigating the catheter with heparinized saline (10 units/ml). The lower strand is tied around the catheter and the catheter further secured with ligatures to several points between the 2 surgical strands to securely anchor the tubing to the muscles of the neck. The catheter is brought subcutaneously to pass through the skin at the back of the neck. This is done to keep the rabbit from chewing on the end and offers a spring action so movement of the animal's neck and shoulders do not place undue strain on the catheter where it is sutured to the vein. Blood samples are collected from the heparinized, indwelling jugular catheter at 5, 15, 30, 60 minutes and 2, 4, 8, 24 and 48 hours after intravenous injection of 200 μCi of Co-57 pepleomycin. The rabbits are kept in metabolic cages (Hoeltge Co., Cincinnati, OH) for collection of 24 and 48 hours urine samples.

CHAPTER 4

COMPUTATIONS

Tissue Distribution Calculations

Data obtained to compare the tumor-seeking abilities of radiolabeled compounds can be approached by two methods. The first method involves x-ray photography of the animal following administration of the radiopharmaceutical. Anesthetized animals are placed under a gamma-camera and images of the distribution of radioactivity in the body are taken at various time intervals. The advantage of this technique is that the results, although qualitative in nature, have direct clinical applications: either a tissue can be visualized or it cannot. In addition, this type of study permits the use of the same animal at various times after injection, thus reducing the cost of the pilot research study. The method also allows for survival of the animal, so that chronic toxicity may be evaluated. Furthermore, quantitation of the data may be obtained by measurement of the intensity of the radiation at various times in regions of interest using a computer online with the gamma-camera. In order to achieve precise quantitation of the data necessary for dosimetric calculations, tissue distribution studies by necropsy must be performed. This involves sacrificing animals at specified times after injection of the radiopharmaceutical, excising the tissues of interest, and quantitating the radioactivity that

these tissues contain by counting them in test tubes in a suitable radiation counting device. The time periods of interest will vary depending on the nature of the study and effective half-life of the radionuclide.

$$\frac{1}{T_{\frac{1}{2}}\text{ (effective)}} = \frac{1}{T_{\frac{1}{2}}\text{ (biological)}} + \frac{1}{T_{\frac{1}{2}}\text{ (physical)}}$$

This study is intended to compare the tumor visualizing ability of ^{57}Cu-bleomycin and 4 analogues by both methods. The types of normal tissue chosen for excision and subsequent analysis were chosen based on radiosensitive tissue (bone marrow and gut), mechanism of action and toxicity of bleomycin. Data gathered in the post-mortem studies were expressed in the following manner:

1) Radioactivity concentration of each tissue as % dose/gram of tissue.
2) Tumor-to-organ ratio, which is defined as,

$$\frac{\%\text{ dose/gram of tumor}}{\%\text{ dose/gram of organ}} .$$

The calculations of percent administered dose and tumor-to-organ ratio were carried out by the computer program GAMCNT (Appendix A), and are expressed as follows:

TAD = (dose x dil x stnda/stndr) - tail

where

TAD = total administered dose in counts per minute

dose = activity of the dose in counts per minute (full dose syringe activity minus empty syringe activity)

stnda = activity of a 1 milliliter aliquot of the diluted standard as counted in the automatic, scintillation well counter (counts/min)

stndr = activity of the undiluted standard (full standard syringe minus the empty standard syringe in counts per minute)

tail = activity still in the tail of the mouse at time of sacrifice and thus not contributing to the dose (counts/minute)

dil = dilution factor

Note:
(all activities are adjusted for background but not for counter deadtime).

$$PADGS = (Act) / (orwt \times TAD)$$

where

PADGS = percent total administered dose per gram sample

Act = organ activity as measured in the automatic, scintillation well counter (counts/minute)

orwt = organ weight (g):

then

$$T/M = PADGS\ (tumor) / PADGS\ (muscle)$$

and

$$T/B = PADGS\ (tumor) / PADGS\ (blood)$$

where

T/M = specific activity of tumor divided by that of muscle

T/B = specific activity of tumor divided by that of blood.

These ratios are the ones calculated in the program GAMCNT. (GAMCNT also calculates other results which were not used in this analysis, hence the subsections DATAD1 and CLLIQ.)

Statistical Analysis

Since the aim of this project was to compare biological distribution of radiolabeled bleomycin and analogues, some statistical test was required to determine whether or not a difference between compounds was significant. The analysis of variance test of significance described in Armitage (1977) is one such test and it was used to compare the compounds on the basis of the tumor-to-muscle and tumor-to-blood ratios. The data are divided into ten groups for the tumor-to-muscle data and into ten groups for the tumor-to-blood data: in each case, there is one group for each time interval (4 hr, 24 hr) and there are five compounds, giving ten groups. The analysis of variance technique compares the time interval groups of one compound with those of the other (between compounds), it compares individual time interval groups in a given compound (between times) and it also compares the cross product of these two. The results of these comparisons are three F ratios which are then used to determine whether or not a significant difference exists for a given comparison. Analysis of variance, in general, is used to determine whether or not two or more groups have been taken from a single population. Then,

for those comparisons that produced a significant difference, a Student's "t"-test is carried out to identify the exact groups which produced the difference. It is theoretically possible to apply the "t"-test to each group initially if it is known beforehand that these comparisons are to be made. However, the analysis of variance test is a more general one. The power of the analysis of variance test of significance lies in the fact that identifiable factors can be separated and tested to see the effect each has on the variation in the sample population. The computations were made using the computer program ANOVA2 (Appendix B).

Statistical analysis of the larger sample size data was performed utilizing the F-distribution, pooled variance estimate, and separate variance estimate on the Cyber-175 (Control Data Corp., Minneapolis, MN) using the SPSS-10 statistical computer program from the University of Pittsburgh. The statistical formulas used as stated in Armitage (1977) and Bailey (1981) are as follows:

1) F-distribution

$$F = \frac{S_1^2}{S_2^2}$$

2) Pooled variance estimate

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{s_p\sqrt{(1/n_1)+(1/n_2)}}$$

$$S_1^2 = \frac{1}{n_1-1} \sum_{i=1}^{n} (x_{1i} - \bar{x}_1)^2$$

$$S_2^2 = \frac{1}{n_2-1} \sum_{i=1}^{n} (x_{2i} - \bar{x}_2)^2$$

$$S_p^2 = \frac{(n_1-1)\ S_1^2 + (n_2-1)\ S_2^2}{n_1 + n_2 - 2}$$

3) Student's "t"-test

$$t = \frac{\bar{x} - \mu}{S/\sqrt{N}}$$

4) Separate variance estimate

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{\sqrt{(S_1^2/n_1)+(S_2^2/n_2)}}$$

$$V = \frac{(S_1^2/n_1 + S_2^2/n_2)^2}{\frac{(S_1^2/n_1)^2}{(n_1-1)} + \frac{(S_2^2/n_2)^2}{(n_2-1)}}$$

There are several assumptions that must be considered when using the analysis of variance technique. The first is that the sample follows a normal distribution. A moderate deviation from this assumption does not have a significant effect on the results, but a large deviation will, especially when there is a borderline significance in the results. Also, it is assumed that the variances of each group should be similar.

The ^{57}Co-pepleomycin blood concentration versus time data for all rabbits were fitted to a multiexponential equation, using a computer program called NONLIN by Metzler (1969). Preliminary parameter estimates

were obtained from a program called Automod by Gomeni and Gomeni (1979): The equation used was

$$\ell n\ C = \ell n\ (A_1 e^{-\alpha t} + A_2 e^{-\beta t} + A_3 e^{-\gamma t})$$

where C is the ^{57}Co-pepleomycin blood concentration at time t after drug administration, A_{1-3} are coefficients, and α, β and γ are first-order elimination rate constants. The plasma half-lives, $T_{\frac{1}{2}}$, were determined from the corresponding rate constants ($r = \alpha, \beta, \gamma$),

$$T_{\frac{1}{2}} = \frac{0.693}{r}$$

The pharmacokinetic parameters were calculated from only the α and β phases of the curve, since comparison by Alberts et al. (1979) between bleomycin and ^{57}Co-bleomycin blood clearance curves suggested that the γ-phase of the ^{57}Co-bleomycin curve was actually due to the existence of free ^{57}Co bound to serum proteins. The area under the curve (CxT) for ^{57}Co-pepleomycin was obtained from

$$CxT = \frac{A_1}{\alpha} + \frac{A_2}{\beta} \quad .$$

The total body clearance (Q_B) and the apparent values of distribution (V_d) for ^{57}Co-pepleomycin were then evaluated from

$$Q_B = \frac{\text{total administered dose (TAD)}}{CxT}$$

and

$$V_d = \frac{TAD}{CxT \cdot \beta} \quad .$$

Fitting of ^{57}Co-Pepleomycin Pharmacokinetic Data

The following sequence of steps was performed to calculate the pharmacokinetic parameters using the NONLIN computer program on the Cyber-175:

(1) Graph the data using semi-logarithmic graph paper.

(2) Decide which type of the equations to use:

(a) IV:

$$c = \sum_{1}^{n} A_i e^{-b_i t}$$

(b) oral:

$$c = \sum_{1}^{n} A_i e^{-b_i t} \quad \text{and} \quad \sum_{1}^{n} A_i = 0$$

(c) infusion:

$$c = \sum_{1}^{n} A_i (1 - e^{-b_i t})$$

(3) Decide a preliminary estimate of n, the number of phases (or exponential terms). This can be easily done by looking at the curves.

(4) Estimate the values of A_i and b_i by using one of the following methods:

(a) Graphic estimation,

(b) AUTOMOD (Gomeni and Gomeni, 1979).

(5) Use NONLIN to fit curves.

(a) Obtain a copy of NONLIN user's manual and familiarize with the input data format and the setup of subroutine DFUNC.

(b) The control cards are:

```
NONLN,BNxxxxxxxx,CM105k,T9.
DISPOSE(PLOT,*PT=30)
ATTACH(NONLIN,ID=HSCHEN)
FTN.
LOAD(LGO)
NONLIN.
7/8/9
```

A copy of the NONLIN program has been compiled and stored as a permanent file under NONLIN, id is HISCHEN. The file will be in computer forever. If the file is not used for a while, it will be moved to disk and will take about 5 minutes to load into memory.

```
SUBROUTINE DFUNC(.....)
.
.
.
.
END
7/8/9
```

Setup of DFUNC depends on the type of equation and on n.

```
data sets
9
6/7/8/9          (pink card)
```

Data format should be consistent with DFUNC.

(c) Change concentration c into ln c.

(d) Run the program and examine the output. If the curve fit is bad, this usually happens when one or two of the parameters reaches the predetermined upper or lower limits, or when most of the points are on one side of the curve. There are several ways to handle the situation:

(i) Change the upper or lower limits for the parameters.

(ii) Change the initial estimates of the parameters.

(iii) Change the equation, by putting in a delay time or by increasing n.

(e) Usually n, the number of exponential terms is determined by the F-test according to Boxenbaum et al. (1974).

Radiation Dosimetry

The radiation dosimetry was calculated by a computer program called DOSE (Appendix C) utilizing data obtained from MIRD pamphlets of Snyder et al. (1975) and Dillman and Vonder Lage (1975). A simplified example of the calculations are as follows:

$$\overline{D}_{(t \leftarrow s)} = \frac{\tilde{A}_s}{m_t} \Delta_i \phi_{(t \leftarrow s)}$$

D = average dose (Rad) to the target organ, t, from the source organ, s.

A = cumulated activity of the radionuclide (μCi-hr) in the source organ, s.

m_t = mass of the target organ, t, for which the dose is to be calculated (grams).

Δ_i = equilibrium absorbed dose constant for the radionuclide $\left(\frac{\text{gram-rad}}{\mu\text{Ci - hr}}\right)$

$\phi_{(t \leftarrow s)}$ = absorbed fraction (dimension-less), fraction of the energy emitted from the source organ, s, that is absorbed in the target organ, t.

The cumulated activity, $\tilde{A}_s$, relates the quantity of activity in the source organ irradiating either the tissue containing the radioactivity or nearby tissues, and the length of time that this activity is present

in the source organ. The tissue that contains the radioactivity is referred to as the source region, denoted by subscript, s. The tissue for which the dose is to be calculated is referred to as the target region, denoted by subscript, t. In many instances, the source and target regions are identical. The equilibrium absorbed dose constant (Δ_i) is the total energy released per disintegration by the radionuclide. The absorbed fraction (ϕ) is the ratio of the energy deposited by the radionuclide in the target organ to that emitted by the source organ.

CHAPTER 5

RESULTS

The yield of ^{57}Co-bleomycin was greater than 99% as determined by thin-layer chromatography. Table 1 presents the chromatography of all radiolabeled analogues with methanol:10% ammonium acetate solution (1:1) used as the solvent. Since the original bleomycin described by Umezawa et al. (1966a) contained at least 11 components, it was no surprise to see multiple radiolabeled peaks in both the phleomycin and tallysomycin thin-layer radiochromatographs. Remers (1979) reported that the antibiotics are isolated from harvested fermentation broth by ion-exchange gradient elution column chromatography. It is virtually impossible to isolate pure individual components by this method. The ^{57}Co-bleomycin R_f values using this thin-layer chromatography system are similar to the R_f values of copper (II) complexes of individual bleomycin components which were determined by Umezawa et al. (1966b). The radiolabeled bleomycinic acid (R_f-0.74) and pepleomycin (R_f-0.64) had one intense peak. Copper (II) chelated bleomycinic acid was reported by Umezawa et al. (1973) to have an R_f of 0.78. A thorough search of the literature revealed no reported R_f values for chelated pepleomycin, but the uv (254 nM) and radiolabeled peak coincided.

The data for the *in vitro* experiments is presented in Figure 4. The curves were not normalized to total counts added at time zero

Table 1. Results of R_f values of radiolabeled (^{57}Co) bleomycin and four analogues using thin-layer chromatography with methanol:10% ammonium acetate solution (1:1) as the solvent.

Bleomycin Analogues	Rf				
	1	2	3	4	5
Bleomycin	0.38	0.68	—	—	—
Bleomycin Acid	0.74	—	—	—	—
Tallysomycin	0.41	0.70	—	—	—
Pepleomycin	0.64	—	—	—	—
Phleomycin	0.19	0.29	0.44	0.65	0.81

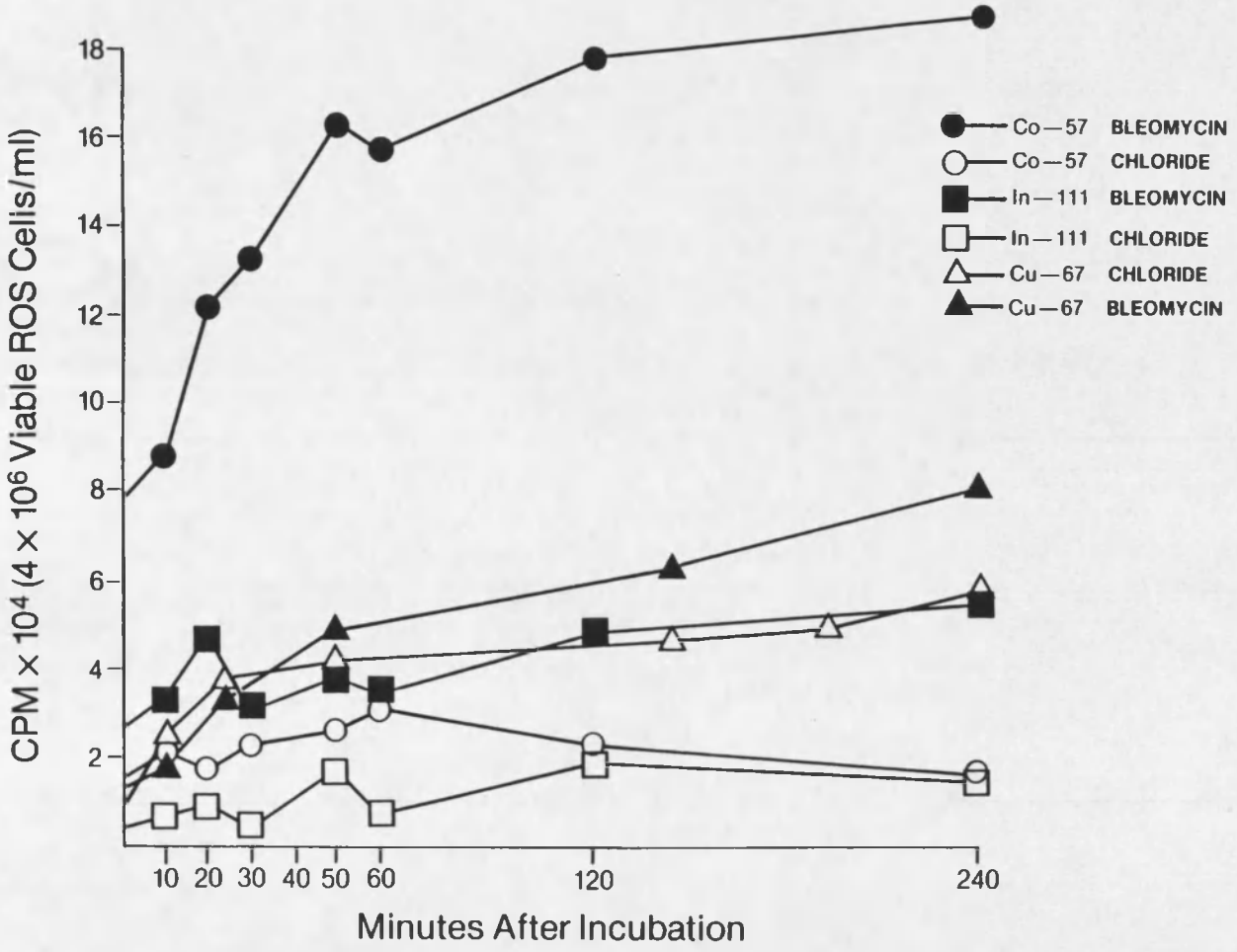


Figure 4. Influence of incubation time on radiolabeled compound uptake by ROS mouse tumor cells.

because of the differences in counting efficiency for the various radionuclides. The relationships between incubation time and radiolabeled uptake did not appear to be linear. There was no cyclic type rise in uptake which would portray some type of interaction at certain phases of the cell cycle. ^{111}In-bleomycin and ^{111}In-chloride demonstrate a 2-fold uptake by the ROS tumor cells at 20 minutes incubation time, but then the radiolabel leaves the cell at 30 minutes and approaches time zero indicating transfer out of the cell. This could indicate instability of the complex and binding to a secondary site. ^{67}Cu-bleomycin and ^{67}Cu-chloride both exhibited a 5.5-fold uptake of radiolabel with incubation time indicating that the uptake of radiolabel is not bleomycin dependent. The ROS tumor cells demonstrate a 2.5-fold increased uptake of the radiolabel over the four hour incubation period with a saturation phenomena observed at two hours, whereas the ^{57}Co-chloride portrays a 2-fold increase in uptake up to one hour and then began to decrease indicating transfer out of the cell.

The results of the tissue distribution studies in mice are given in Tables 2-9. Tables 2 and 3 present results from the biological distribution ^{57}Co-bleomycin and analogs at four and twenty-four hours in AKD_2F_1 mice with ROS tumors; while Tables 8 and 9 show the results from ^{57}Co-bleomycin and ^{57}Co-pepleomycin using a larger number of AKD_2F_1 mice at twenty-four hours, respectively. The highest absolute tumor uptake (% dose/g) at four hours was obtained with radiolabeled bleomycin followed by tallysomycin, bleomycinic acid, pepleomycin and phleomycin. At twenty-four hours the descending order with respect

Table 2. Tissue distribution of radiolabeled (^{57}Co) bleomycin and four analogues at four hours after intravenous injection in AKD_2 mice bearing (ROS) tumor.

	% of Injected Dose Remaining at 4 Hours (Mean ± SD of 5 Animals)				
Tissue	Bleomycin	Pepleomycin	Phleomycin	Tallysomycin	Bleomycinic Acid
Lung	.287 ± .091	.239 ± .146	.211 ± .179	1.34 ± .977	.088 ± .040
Liver	.680 ± .299	.975 ± .608	4.936 ± 3.667	15.81 ± 6.58	.147 ± .066
Spleen	.417 ± .223	.297 ± .140	.552 ± .508	3.163 ± 1.142	.09 ± .065
Kidney	2.447 ± 1.531	2.449 ± 1.702	2.203 ± 1.58	26.75 ± 16.37	1.809 ± 1.205
Heart	.062 ± .025	.060 ± .025	.068 ± .052	.487 ± .439	.036 ± .021
Muscle	.077 ± .018	.060 ± .049	.062 ± .057	.241 ± .146	.109 ± .187
Gut	.200 ± .131	.184 ± .125	.392 ± .349	.585 ± .353	.169 ± .200
Bone	.265 ± .229	.195 ± .200	.235 ± .180	1.766 ± 1.025	.176 ± .234
Blood	.052 ± .016	.044 ± .021	.058 ± .053	.144 ± .098	.018 ± .010
Tumor	2.618 ± 1.821	1.488 ± 1.431	.850 ± 1.038	2.123 ± 1.598	1.948 ± 1.249

Table 3. Tissue distribution of radiolabeled (^{57}Co) bleomycin and four analogues at twenty-four hours after intravenous injection in AKD_2 mice bearing (ROS) tumor.

	% of Injected Dose Remaining at 24 Hours (Mean ± SD of 5 Animals)				
Tissue	Bleomycin	Pepleomycin	Phleomycin	Tallysomycin	Bleomycinic Acid
Lung	.301 ± .109	.101 ± .060	.500 ± .225	.310 ± .116	.023 ± .008
Liver	1.420 ± .790	.777 ± .445	12.54 ± 4.24	6.077 ± 1.788	.044 ± .018
Spleen	.338 ± .158	.186 ± .135	4.229 ± 1.978	1.621 ± 1.263	.034 ± .017
Kidney	2.476 ± 1.154	.860 ± .429	9.100 ± 3.595	7.089 ± 2.115	.271 ± .105
Heart	.136 ± .078	.054 ± .036	.138 ± .075	.128 ± .035	.013 ± .005
Muscle	.160 ± .102	.022 ± .009	.063 ± .035	.062 ± .027	.006 ± .002
Gut	.314 ± .176	.090 ± .057	1.678 ± .850	.212 ± .081	.034 ± .025
Bone	.176 ± .078	.072 ± .033	.892 ± .347	.429 ± .114	.019 ± .007
Blood	.106 ± .090	.029 ± .018	.021 ± .006	.098 ± .130	.005 ± .003
Tumor	3.864 ± 2.796	1.850 ± 1.533	.986 ± .478	1.558 ± 1.235	.258 ± .189

Table 4. Tumor to organ ratios of radiolabeled (^{57}Co) bleomycin and four analogues at four hours after intravenous injection in AKD_2F_1 mice bearing (ROS) tumor.

Tumor/Organ Ratio	Bleomycin	Pepleomycin	Phleomycin	Tallysomycin	Bleomycinic Acid
Lung	9.12	6.23	4.03	1.67	21.91
Liver	3.85	1.65	0.17	0.13	13.26
Spleen	6.28	5.52	1.54	0.67	21.66
Kidney	1.07	0.54	0.39	0.08	1.08
Heart	42.20	24.80	12.50	4.36	54.16
Muscle	34.00	24.80	13.70	8.80	17.88
Gut	13.10	8.11	2.17	3.63	11.54
Bone	9.88	7.60	3.62	1.20	11.08
Blood	49.20	33.80	14.60	14.74	108.33

Table 5. Tumor to organ ratios of radiolabeled (^{57}Co) bleomycin and four analogues at twenty-four hours after intravenous injection in AKD_2F_1 mice bearing (ROS) tumor.

Tumor/Organ Ratio	Bleomycin	Pepleomycin	Phleomycin	Tallysomycin	Bleomycinic Acid
Lung	14.10	18.32	1.97	5.02	11.22
Liver	1.89	2.38	0.08	0.26	5.86
Spleen	12.44	9.95	0.23	0.96	7.59
Kidney	1.37	2.15	0.11	0.22	0.92
Heart	23.50	34.26	7.14	12.17	19.84
Muscle	29.50	61.10	15.65	25.12	41.93
Gut	12.09	20.55	0.59	7.35	7.59
Bone	19.23	25.69	1.10	3.63	13.58
Blood	35.90	65.40	46.95	15.90	57.77

Table 6. Results of student's t-test of comparison of tumor-to-muscle ratios of radiolabeled (^{57}Co) bleomycin and four analogues at four and twenty-four hours after intravenous injection.

Compound X

Compound Y better than: Agent	Bleo mix 24 hr	Bleo mix 4 hr	Phleo 4 hr	Phleo 24 hr	Tally 4 hr	Tally 24 hr	Bleo acid 4 hr	Bleo acid 24 hr	Pep 4 hr	Pep 24 hr	f of y > x
Bleo mix 24 hr			°	°	°	°			•		5
Bleo mix 4 hr			°								1
Phleo 4 hr											0
Phleo 24 hr											0
Tally 4 hr											0
Tally 24 hr			°								1
Bleo acid 4 hr			°	°	°	°			°		5
Bleo acid 24 hr			°	°	°						3
Pep 4 hr											0
Pep 24 hr		°	°	°	°	°			°		6

Table 7. Results of student's t-test of comparison of tumor-to-blood ratios of radiolabeled (^{57}Co) bleomycin and four analogues at four and twenty-four hours after intravenous injection.

Compound X

Compound Y better than	Bleo mix 24 hr	Bleo mix 4 hr	Phleo 4 hr	Phleo 24 hr	Tally 4 hr	Tally 24 hr	Bleo acid 4 hr	Bleo acid 24 hr	Pep 4 hr	Pep 24 hr	f of y>x
Bleo mix 24 hr			∘		∘	∘			∘		4
Bleo mix 4 hr			∘			∘			∘		3
Phleo 4 hr											0
Phleo 24 hr						∘			∘		2
Tally 4 hr											0
Tally 24 hr											0
Bleo acid 4 hr	∘		∘		∘	∘			∘		5
Bleo acid 24 hr			∘								1
Pep 4 hr											0
Pep 24 hr			∘			∘			∘		3

Table 8. Tissue distribution of radiolabeled bleomycin at twenty-four hours after intravenous injection in AKD_2F_1 mice bearing (ROS) tumor.

Tissue	% Administered Dose/Gram of Tissue	Tumor: Organ Ratio
Lung	0.1071 ± 0.062	13.11 ± 5.39
Liver	0.4620 ± 0.273	3.21 ± 1.36
Spleen	0.1842 ± 0.099	7.25 ± 2.31
Kidney	0.7822 ± 0.606	1.73 ± 0.44
Heart	0.0803 ± 0.108	24.74 ± 13.57
Muscle	0.0269 ± 0.014	52.51 ± 28.56
Skin	0.1659 ± 0.160	11.26 ± 6.85
Gut	0.0995 ± 0.062	14.01 ± 5.83
Bone	0.0834 ± 0.100	25.95 ± 23.79
Blood	0.0259 ± 0.022	61.02 ± 28.85
Tumor	1.2148 ± 0.519	—

Table 9. Tissue distribution of radiolabeled pepleomycin at twenty-four hours after intravenous injection in AKD_2F_1 mice bearing (ROS) tumor.

Tissue	% Administered Dose/Gram of Tissue	Tumor: Organ Ratio
Lung	0.1125 ± 0.055	20.66 ± 12.30
Liver	0.8532 ± 0.376	2.58 ± 1.08
Spleen	0.2448 ± 0.180	10.88 ± 6.10
Kidney	0.9276 ± 0.413	2.54 ± 1.89
Heart	0.0703 ± 0.080	44.21 ± 27.72
Muscle	0.0277 ± 0.012	86.27 ± 51.33
Skin	0.1983 ± 0.181	24.22 ± 44.32
Gut	0.1182 ± 0.059	20.47 ± 11.72
Bone	0.0865 ± 0.043	27.20 ± 15.12
Blood	0.0315 ± 0.031	102.00 ± 70.61
Tumor	2.0182 ± 0.780	—

to absolute tumor concentration was bleomycin, pepleomycin, tallysomycin, phleomycin, and bleomycinic acid. The distribution in other tissues indicated that the biologic behavior of the radiolabeled analogs was different; the liver and spleen concentrations for phleomycin and tallysomycin were significantly higher ($p < 0.05$) than that for bleomycin, bleomycinic acid and pepleomycin at four and twenty-four hours. The uptake of ^{57}Co-tallysomycin in the liver decreased from four to twenty-four hours, while ^{57}Co-phleomycin increased over this time span. Blood levels of all the radiolabeled compounds were very low suggesting rapid tissue distribution and excretion of these agents. Low muscle and heart uptake of all the radiolabels suggests little movement into these cells. The high bone uptake (this sample includes the bone marrow) for tallysomycin and phleomycin indicated that these agents would not be ideal radiopharmaceuticals, because bone marrow is the most radiosensitive tissue. Kidney concentration is high because this is the primary route of excretion of cobalt-bleomycin, but some binding to the kidney is demonstrated by the increasing concentrations of tallysomycin and phleomycin at twenty-four hours. The four-hour lung uptake of tallysomycin was highest (1.34 ± 0.977% dose/g) and the lowest was bleomycinic acid (0.088 ± 0.040% dose/g). The gut concentration of tallysomycin and phleomycin was the highest suggesting some excretion of the radiolabel into that organ, probably via the biliary route, as both are concentrated highly in the liver.

Tables 4 and 5 give a comparison of ratios of percent administered dose/gram for tumor to lung, liver, spleen, kidney, heart, muscle, gut,

bone and blood in the AKD_2F_1 mice bearing ROS tumors for ^{57}Co-bleomycin and analogues. The descending order of tumor-to-blood ratios for the radiolabeled compounds at four hours were bleomycinic acid, bleomycin, pepleomycin, tallysomycin, and phleomycin, respectively. The large change in order of tumor-to-blood ratios at twenty-four hours (pepleomycin > bleomycinic acid > phleomycin > bleomycin > tallysomycin) probably reflects the variation in rate of clearance from the blood. Nevertheless, the tumor-to-blood ratios for all agents exceeded 10:1 at four hours and 15:1 at 24 hours, with tallysomycin demonstrating the lowest ratios at both times. The tumor-to-muscle ratios for all radiolabeled compounds portrayed a maximum at twenty-four hours, except for bleomycin, which essentially did not change. The absolute tumor uptake of bleomycin increased from four to twenty-four hours, but the % administered dose/g in the blood also increased. The low tumor-to-organ (lung, liver, spleen, gut) ratios for phleomycin and tallysomycin indicate they are not suitable substitutes for ^{57}Co-bleomycin.

Tables 6 and 7 compare the effectiveness of all five radiolabeled compounds at four and twenty-four hours distribution to predict which agent is better by the Student's "t"-test described in Bailey (1981). The parameters used were tumor-to-blood and tumor-to-muscle ratios of % administered dose/g of tissue, which are the best indicators of tumor visualization. Table 6 demonstrates the results of comparing tumor-to-muscle ratios and suggests that a better agent or time of localization is bleomycin at 24 hours, pepleomycin at 24 hours or bleomycinic acid at 4 hours. Table 7 compares the tumor-to-blood ratios which again suggests

bleomycinic acid is better localized at 4 hours, and bleomycin or pepleomycin at 24 hours. No additional bleomycinic acid for further studies was obtainable because of expense (i.e., $1000/mg) and the low yield of preparation of bleomycinic acid (0.8%) from bleomycin A_2. This suggested further studies in a larger group of animals comparing ^{57}Co-pepleomycin with ^{57}Co-bleomycin.

Tables 8 and 9 illustrate the biological distribution and tumor-to-organ ratios of ^{57}Co-bleomycin and ^{57}Co-pepleomycin in a larger group of animals at twenty-four hours after intravenous injection. The absolute tumor uptake (% dose/g) of Co-57 pepleomycin was 1.66 greater than Co-57 bleomycin. In contrast, in the smaller group animal study, Co-57 pepleomycin tumor uptake was significantly less (almost 0.5) than the Co-57 bleomycin tumor uptake. These data demonstrate the need for a larger sample size for biological distribution studies of radiopharmaceuticals.

Tables 10 and 11 represent the statistical comparison of absolute organ uptake (% dose/g) and tumor-to-organ ratios for ^{57}Co-bleomycin versus ^{57}Co-pepleomycin. According to Schefler (1969) the F-values of one or less are automatically nonsignificant, but any F-value larger than one should be checked by using the critical values of F table (Schefler, 1969, p. 218). The absolute tumor uptake (% dose/g) is significantly different ($p < 0.1$). In contrast, the pooled and/or separate variance estimate of the absolute tumor uptake (% dose/g) was significantly different at the $p < 0.0005$ level. ^{57}Co-pepleomycin had a significantly higher ($p < 0.05$) tumor-to-organ ratio for all organs but the liver when using the F-distribution. In addition, the tumor-to-organ ratio was

Table 10. Statistical comparison of % administered dose/g of tissue (11) distribution of radiolabeled (^{57}Co) bleomycin and pepleomycin at twenty-four hours after intravenous injection in AKD_2F_1 mice bearing (ROS) tumor.

	F-Distribution		Pooled Variance Estimate			Separate Variance Estimate		
Organ	F Value	2-Tail Prob.	T Value	Degrees of Freedom	2-Tail Prob.	T Value	Degrees of Freedom	2 Tail Prob.
Lung	1.30	0.558	−0.30	42	0.762	−0.31	41.94	0.761
Liver	1.90	0.145	−3.97	42	<0.0005	−3.92	36.21	<0.0005
Spleen	3.34	0.007	−1.40	42	0.169	−1.36	30.37	0.183
Kidney	2.15	0.089	−0.92	42	0.362	−0.94	38.97	0.354
Heart	1.83	0.179	0.35	42	0.731	0.35	40.33	0.727
Muscle	1.39	0.467	−0.21	42	0.835	−0.21	41.80	0.833
Skin	1.28	0.579	−0.62	41	0.536	−0.62	38.34	0.540
Gut	1.12	0.810	−0.99	40	0.327	−1.00	39.25	0.325
Bone	5.34	<0.0005	−0.13	42	0.896	−0.14	30.54	0.893
Blood	2.01	0.113	−0.69	42	0.496	−0.68	35.59	0.503
Tumor	2.26	0.066	−4.06	42	<0.0005	−3.98	34.34	<0.0005

Table 11. Statistical comparison of tumor-to-organ ratios (10) for radiolabeled (^{57}Co) bleomycin and pepleomycin at twenty-four hours after intravenous injection in AKD_2F_1 mice bearing (ROS) tumor.

Tumor:	F-Distribution		Pooled Variance Estimate			Separate Variance Estimate		
Organ Ratio	F Value	2-Tail Prob.	T Value	Degrees of Freedom	2-Tail Prob.	T Value	Degrees of Freedom	2 Tail Prob.
Lung	5.22	<0.0005	−2.68	42	0.011	−2.59	26.86	0.015
Liver	1.58	0.310	1.68	42	0.100	1.70	41.27	0.097
Spleen	6.99	<0.0005	−2.65	42	0.011	−2.56	25.18	0.017
Kidney	18.44	<0.0005	−2.01	42	0.051	−1.92	21.98	0.067
Heart	4.17	0.002	−3.00	42	0.005	−2.92	28.47	0.007
Muscle	3.23	0.009	−2.73	42	0.009	−2.66	30.68	0.012
Skin	41.83	<0.0005	−1.39	41	0.173	−1.29	19.79	0.210
Gut	4.04	0.002	−2.33	40	0.025	−2.19	25.25	0.038
Bone	2.48	0.046	−0.21	42	0.838	−0.21	37.66	0.835
Blood	5.99	<0.0005	−2.56	42	0.014	−2.48	26.01	0.020

significantly higher ($p < 0.05$) for all organs except for liver, kidney, skin and bone when utilizing the pooled and/or separate variance estimate.

Table 12 presents the pharmaceutical parameters and urinary excretion data for ^{57}Co-pepleomycin after intravenous administration. The arithmetic means of the individual rabbit parameters appear at the bottom. Co-57 pepleomycin disposition data showed rapid plasma disappearance in the alpha phase ($T_{\frac{1}{2}}$-0.88 hours) and greater than 80 percent excretion in the urine in the first 48 hours. The terminal phase plasma half-life (Beta) was extremely long, but the amount of Co-57 present during this phase was very small (less than 2% of administered dose). Alberts et al. (1979) have demonstrated in rabbits using ^{57}Co-bleomycin and ^{57}Co-chloride that free ^{57}Co accounts for the tail end of both terminal phases. The pharmacokinetic parameters were similar to those reported by Alberts et al. (1979) for ^{57}Co-bleomycin.

Figure 5 portrays the scintiphotos of four rabbits obtained with a gamma camera with whole body imaging table (Searle Radiographics Pho/Gamma IV) at 24 hours after intravenous injection of 200 μCi of ^{57}Co-bleomycin, pepleomycin, phleomycin and tallysomycin. The tumor (V-2 carcinoma) in the right flank is clearly visualized with all agents. The kidneys and bladder are localized with all radiolabeled compounds, and a faint liver image is also evident on the ^{57}Co-bleomycin and ^{57}Co-pepleomycin images. ^{57}Co-tallysomycin and ^{57}Co-phleomycin demonstrate significant liver, spleen and bone uptake as previously demonstrated by the quantitative data in mice.

Table 12. Pharmacokinetic parameters of ^{57}Co-pepleomycin in normal NZW rabbits.

Rabbit #	A_1 (Ncpm/ml)	T½-α (hr)	A_2 (Ncpm/ml)	T½-β (hr)	Vd (Liters)	Q_8 (ml/min)	48-Hr Urinary Excretion (% Administered Dose)
1	276330	0.592	3379	82.2	23.0	194	93.2
2	147005	0.636	1454	293.1	58.2	138	85.4
3	196936	0.907	1356	139.2	70.4	206	90.7
4	157406	0.714	1163	—[a]	—	—	77.6
5	45861	1.553	1331	75.8	25.0	229	89.0
Mean[b]	—	0.880 ± 0.397	—	147.6 ± 159.9	44.1 ± 23.8	191.7 ± 38.7	87.2 ± 6.06

a—Parameter could not be determined
b—Mean: Pharmacokinetic parameters ± SD

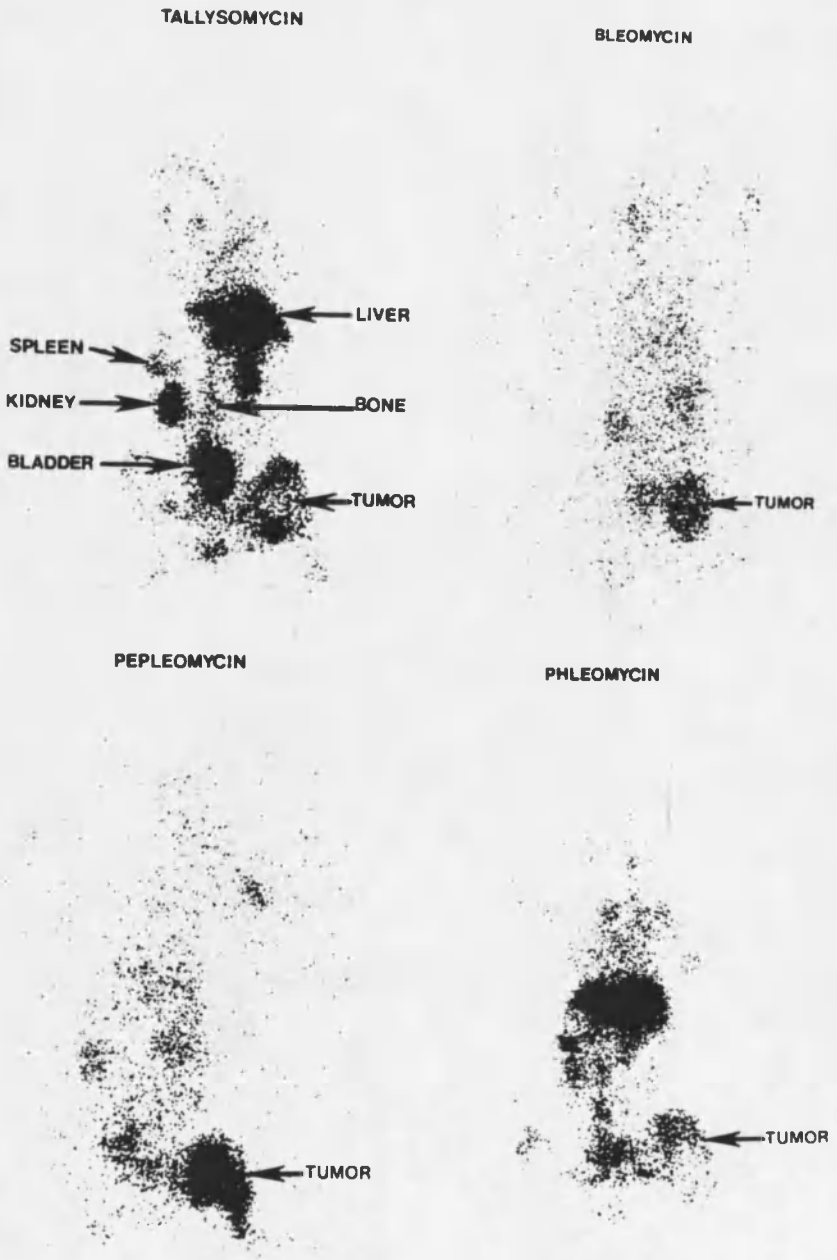


Figure 5. Comparison by scintiphotography at 24 hours after intravenous injection of Co-57 (200 μCi) radiolabeled tallysomycin, bleomycin, pepleomycin and phleomycin in NZW rabbits bearing V-2 carcinoma in the right hind leg.

An estimation of the whole body absorbed dose following intravenous administration of 1 mCi of ^{57}Co-pepleomycin to a 70 kg patient was calculated to be 0.10 Rads. Several assumptions were made in the calculation including a 173-hour effective half-life for 20% of the dose taken from the paper by Rasker et al. (1975) for the long term component of ^{57}Co-bleomycin. There were some problems in the calculations performed by this computer program. The major problem was that the program does not take into consideration that the radiopharmaceutical may have its own associated half-life in each tissue, with a given half-life for a tissue being different from the others. Therefore, what is entered in the category of tissue concentration is not representative throughout time. Adequate animal data have not been taken to obtain accurate dosimetry calculations and this must be done prior to clinical evaluation of this radiopharmaceutical. Anzai et al. (1974) have reported a 60-90 day effective half-life for the terminal phase of ^{57}Co-bleomycin, which would increase the whole body dose to almost 3 Rads. However, the majority of investigators (Rasker et al., 1975; Grove, Eckelman, and Reba, 1974; Nouel et al., 1974) report a total body radiation absorbed dose of less than 0.5 Rads.

CHAPTER 6

DISCUSSION

The in vitro experiments performed (Figure 4) clearly demonstrated that bleomycin facilitates the uptake of ^{57}Co into the ROS tumor cell when compared to the other radiolabels. In addition, the radiolabel does not leave the cell and label a secondary binding site. Bleomycin does not facilitate ROS tumor cellular uptake of ^{67}Cu, because ^{67}Cu-chloride was taken up as well as ^{67}Cu-bleomycin, suggesting that the ^{67}Cu is removed from the bleomycin prior to entering the cell, and is bound to a secondary binding site (i.e., ceruloplasmin), prior to crossing the cell membrane. Both ^{111}In-Bleomycin and ^{111}In-Chloride demonstrated an increase in uptake by the ROS tumor cells, but a decrease was observed after 20 minutes indicating some type of equilibrium reaction with a molecule in the media (i.e., transferrin). Experiments were not performed to determine if this increased ROS tumor cell uptake was due to adsorption to the cell membrane or incorporated into the cell after incubation. Fujimoto (1974) demonstrated by autoradiography that ^{14}C-bleomycin was adsorbed to the surface of the cell membrane of Ehrlich ascites mouse tumor cells after two hours incubation. The radiolabel was incorporated into the cells at four hours and was located mainly on the nuclear membrane. Attempts to duplicate his study using ^{57}Co-bleomycin as the radiolabel with the ROS tumor cell have not been

successful at the present time. It is likely that the ROS tumor cell membrane is very fragile and ruptures very readily upon smearing on glass slides. In addition, ^{57}Co is not a beta emitter so exposure is based on internal conversion electrons where the mean number/disintegration is low for this radionuclide. Consequently, large doses of Co-57 are necessary to obtain an adequate activation of the silver bromide crystals in the photographic emulsion.

The results from the in vivo distribution studies of these radiolabeled bleomycin analogs after further study with a larger sample size suggested that ^{57}Co-pepleomycin is the most useful compound for diagnostic imaging. The radiolabeled compound showed high absolute tumor uptake (2.0182 ± 0.780% dose/g) versus ^{57}Co-bleomycin (1.2148 ± 0.519% dose/g). In addition it produced high tumor-to-non-tumor ratios (Tumor/Muscle: 86.27 ± 51.33; Tumor/Blood: 102.00 ± 70.61) when compared to ^{57}Co-bleomycin (Tumor/Muscle: 52.51 ± 28.56; Tumor/Blood: 61.02 ± 28.85). The tumor concentration of ^{57}Co-pepleomycin did not appear to be blood concentration related, because the tumor-to-blood ratios were not similar and the absolute tumor concentration increased with time. The number of animals studied using ^{57}Co tallysomycin, phleomycin, and bleomycinic acid was considerably less than necessary to give a critical statistical evaluation of the data. However, the increased lung, liver, spleen, and bone marrow uptake of ^{57}Co-tallysomycin and ^{57}Co-phleomycin was real, as demonstrated by both the quantitative data in mice and qualitative data in rabbits. This result suggests that larger sample size would be futile. Further evaluation of ^{57}Co-bleomycinic acid was

impossible due to lack of a source of supply, high cost and low yield on preparation. In addition, the absolute tumor uptake at 24 hours after intravenous injection was very low (0.258 ± 0.189% dose/g) when compared to the other radiolabeled compounds.

Errors in Measurement

The major defect in this study and the one that most influences the statistical analysis is the large variation in the data obtained, which is demonstrated by the large standard deviation in the % dose/g for most of the organs. Several factors are considered that may have caused the wide range of values in each group.

Intravenous injection in mice is not a relatively simple procedure. Because of the small volume used (0.2 ml), loss of even a drop of radioactivity introduces a large error into the measurement. When administering a radiopharmaceutical by this method it is generally indicated to survey the injection site with a Geiger-Meuller counter to ascertain that the injection was indeed performed intravenously and not subcutaneously into the tail tissue. This procedure is an art and takes considerable practice to develop the technique. In addition, the residual volume left in the needle must be accounted for as different gauge needles retain varying volumes. A technique using the surgical approach to expose a large deep vein (i.e., femoral or jugular vein) which can be injected, may eliminate the necessity of monitoring the injection site. However, this method limits the number of animals to be studied due to time constraints.

Biologic variability can be considerable, even using the same strain, age, weight and sex of the animal. Factors such as renal and liver disease affect the biological distribution of a radiopharmaceutical. The radiopharmaceutical injected must represent a single chemical form of the radiolabeled compound, since it will be the radionuclide which is monitored. In vivo metabolism of the radiopharmaceutical can be operative in five different ways: 1) loss of radioactive label with remainder of the molecule left intact, 2) loss of radiolabel concomitant with degradation of the molecule, 3) hydrolysis or enzymatic cleavage of the molecule with radiolabel retention, 4) metabolic utilization of the molecule as a whole, and 5) excretion of the nonmetabolized molecule.

Since tumor size can vary widely throughout a study, this can be a possible source of error. Any animal with a grossly necrotic tumor (greater than 50% of the tumor) was not used in this study because the radiopharmaceutical only concentrates in viable tumor cells. Thus, the relationships between tumor weight and tumor uptake could present a noticeable variation in the data.

Error in sample radioactivity could be introduced if sample volumes are too large for the well counter (geometry error). Another possible sample error is in the counting statistics, but the radioactivity in the samples was large enough that errors in counting were less than five percent.

Error in tissue weights could be a large factor, because all were wet weight samples. Water is evaporating from the sample even while weighing them, as evidenced by decrease in weight with time. Thus, all tissues should be weighed immediately after removal from the animal.

Consequently, the reliability of the results is only as good as the accuracy and precision of the data obtained. Over 150 mice and 20 rabbits were utilized in this study, but only 59 mice and 9 rabbits provided useful data, due to excessive and no tumor growth, incomplete collection of urine samples, accidental death by anesthesia and surgery, and poor intravenous injections. However, it is assumed that the final results obtained are valid since there are no borderline differences in the final statistical evaluation of the data.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

In summary, the biological behavior of ^{57}Co-bleomycin with 4 analogues was compared. It was found that ^{57}Co-pepleomycin accumulated in a mouse tumor model, and the concentration remained high for at least one day, while the blood concentration decreased with a half-life of less than one hour (0.88 hr). Very favorable tumor-to-blood and tumor-to-muscle ratios were obtained, which made it possible for a rabbit tumor to be distinctly visualized scintigraphically 24 hours after intravenous injection. The ^{57}Co-pepleomycin was radiochromatographically pure and appears to behave similarly to ^{57}Co-bleomycin in mouse and rabbit tissues.

The absolute tumor uptake in the mouse of ^{57}Co-pepleomycin 24 hours after intravenous administration was twice that of ^{57}Co-bleomycin. The radiolabeled compound possesses many characteristics which are ideal for external localization of tumors. The radionuclide (^{57}Co) has a principal gamma emission of 122 keV for good resolution with current gamma-cameras. Approximately 90 percent of the administered dose is excreted in the urine within the first 48 hours. Although the physical half-life of the radiolabel is long, the biological half-life is short for the majority of the dose, and the calculated radiation levels to humans are within permissable levels, since beta or position emission is absent. The cobalt chelate of pepleomycin is extremely stable,

chemically. It is a homogeneous second generation analog of bleomycin, hence, the variability and potential radiolabeled impurities encountered with ^{57}Co-bleomycin are completely avoided. These results have been sufficiently encouraging that future clinical trials with ^{57}Co-pepleomycin will be initiated in patients after more pharmacokinetic data is obtained for more accurate radiation dosimetry calculations. Because ^{57}Co has a long half-life (270 days) and it is excreted in the urine, prolonged storage of excreta for good radiation safety is required. Therefore, widespread use outside of large clinical centers is not expected. Pilot studies to radiolabel and determine biological distribution with shorter half-life radionuclides (^{99m}Tc, ^{111}In) have met with failure due to poor in vivo stability of the complex. With the recent advent of efficient instruments for imaging position-emitting radionuclides, it may be worthwhile to label pepleomycin with ^{55}Co ($T_{1/2}$ = 18.2 hours) as an alternative to the long-lived ^{57}Co. The results of this study suggest further evaluation of other radiolabeled analogs of bleomycin for tumor localization.

APPENDIX A

GAMCNT COMPUTER PROGRAM

```
C*******************************************************************************
C*******************************************************************************
C       THIS PROGRAM WAS ADAPTED FOR THE RADIOLOGY VAX-11 COMPUTER
C       BY JAMES A. HENRICKS.  THE AUTHOR OF THE ORIGINAL PROGRAM
C       WAS ROGER TOKARS AND LATER ALTERATIONS WERE MADE BY
C       STANLEY READ AND JOE CHEN.
C                                          JAH  2/23/79
C            THIS PROGRAM CALCULATES THE VALUES OF THE PERCENT
C       ADMINISTERED DOSE PER GRAM SAMPLE(PADGS) FOR 16 ORGANS
C       OR TISSUES.  IT ALSO CALCULATES THE TUMOR-TO-ORGAN(OR
C       TISSUE) RATIOS FROM THE "PERCENT ADMINISTERED DOSE PER
C       GRAM SAMPLE" VALUES.
C            INPUT DATA ARE THE ORGAN(OR TISSUE) WEIGHTS AND AC-
C       TIVITIES (COUNTS PER MINUTE), AND STANDARD AND DOSE SYR-
C       INGE ACTIVITIES.  THIS PROGRAM CAN BE USED FOR
C       ANY RADIOISOTOPE DISTRIBUTION STUDY WHERE TISSUE SAMPLES
C       ARE TAKEN.  IF THE STUDY IS WITH MICE OR RATS, THE NUM-
C       BER OF COUNTS IN THE TAIL SHOULD ALSO BE ENTERED.
C       THIS PROGRAM CAN BE USED WITH DATA FROM A GAMMA OR LIQUID
C       SCINTILLATION COUNTER.
C            A SUBPROGRAM (CALLIQ) IS USED IN CONJUNCTION WITH
C       DATA FROM A LIQUID SCINTILLATION COUNTER.  A BLOCK
C       DATA FILE (DATAD) CONTAINS DATA USED TO SCALE THE RESULTS
C       TO HUMANS.
C*******************************************************************************
C
C       LIST OF VARIABLES:
C              AH- DUMMY VARIABLE USED IN SWITCHING TARE AND STARE
C           AMUSC- DUMMY VARIABLE, NUMERICALLY EQUIVALENT
C                    TO PADGS(MUSCLE)
C           BLOOD- DUMMY VARIABLE NUMERICALLY EQUIVALENT TO
C                    PADGS(BLOOD)
C               I- INDEXING VARIABLE FOR A DO LOOP
C           IDENT- INDEX VALUE USED TO IDENTIFY A PARTICULAR
C                    ORGAN (1=LUNG, 2=LIVER)
C          IFCHNG- INTEGER VARIABLE USED TO DETERMINE
C                    WHETHER OR NOT ANY DATA IS TO BE CHANGED.
C             IJJ- INDEXING VARIABLE FOR A DO LOOP
C          ISTAND- INDEXING VARIBLE EQUIVALENT TO THE NUMBER
C                    OF STANDARDS USED
C           IZZZZ- INTEGER VARIBLE USED TO DETERMINE WHETHER
C                    OR NOT THE LIQUID SCINTILLATION
C                    PROGRAM IS TO BE USED
C              J- INDEXING VARIABLE FOR A DO LOOP
C             JJ- INDEXING VARIABLE FOR A DO LOOP
C            JJJ- INDEXING VARIABLE FOR AN IMPLIED DO LOOP
C            JKL- INDEXING VARIABLE FOR A DO LOOP
C              K- INDEXING VARIABLE FOR A DO LOOP
C             KT- DUMMY VARIABLE FOR ACT(J)
C              L- INDEXING VARIABLE FOR A DO LOOP
C          MSTAKE- INTEGER VARIABLE USED TO DETERMINE WHETHER
C                    OR NOT DATA IS TO BE CHANGED
C             NR- NUMBER OF ANIMALS IN THE STUDY FOR A GIVEN
C                    STANDARD
C             ORN- THE NUMBER OF TISSUE SAMPLES (16)
C            ORWT- TOTAL ORGAN WEIGHT (ESTIMATE GIVEN BY PROGRAM)
C          PADGHW- PERCENT ADMINISTERED DOSE PER GRAM HUMAN
C                    WEIGHT
C           PADGS- PERCENT ADMINISTERED DOSE PER GRAM SAMPLE
C          PADPBW- PERCENT ADMINISTERED DOSE PER PERCENT BODY
C                    THAT IS ORGAN
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```
C              PADS- PERCENT ADMINISTERED DOSE PER SAMPLE
C               TDB- VALUE OF THE RATIO OF TUMOR TO BLOOD
C             TUMOR- PADGS(TUMOR)
C               WTZ- NET SAMPLE WEIGHT
C               ZDB- VALUE OF THE RATIO OF TUMOR TO MUSCLE
C*******************************************************************************
C
C       LIST OF ARRAYS:
C                 ACT- ORGAN(OR TISSUE) ACTIVITY
C               ASYRC- NET ACTIVITY OF EMPTY STANDARD SYRINGE (CPM)
C               ASYRE- ACTIVITY OF EMPTY STANDARD SYRINGE (CPM)
C               ASYRF- ACTIVITY OF FULL STANDARD SYRINGE (CPM)
C                 BGD- BACKGROUND
C               CALIB- DATA BLOCK VARIABLE USED WITH LIQUID
C                        SCINTILLATION PROGRAM
C                DATE- ALPHANUMERIC VARIABLE FOR DATE, DISTRIBUTION
C                        TIME, ETC.
C                  DF- DILUTION FACTOR
C                  II- INDEX VALUE USED TO IDENTIFY AN ORGAN FOR THE
C                        LIQUID SCINTILLATION PROGRAM
C                   N- DATA BLOCK VARIABLE FOR THE ALPHANUMERIC ORGAN
C                        NAMES
C                PMBC- PERCENT MEAN BODY CONCENTRATION
C              PPADGS- EQUIVALENT TO PADGS
C               PTADT- PERCENT ADMINISTERED DOSE IN TAIL
C                RADP- ALPHANUMERIC VARIABLE FOR THE RADIOISOTOPE AND
C                        OTHER PERTINENT INFORMATION
C                 RAT- DATA BLOCK VARIABLE FOR THE FRACTION OF ORGAN
C                        WEIGHT TO TOTAL BODY WEIGHT
C               RATIO- VALUES OF TUMOR-TO-ORGAN (OR TISSUE) RATIOS
C               SCTSM- COUNTS OF DILUTED STANDARD IN AUTOMATIC WELL
C                        COUNTER
C                SPEC- ALPHANUMERIC VARIABLE FOR THE ANIMAL SPECIES
C                        AND OTHER PERTINENT INFORMATION
C               SSYRC- NET DOSE ACTIVITY (CPM)
C               SSYRE- ACTIVITY OF EMPTY DOSE SYRINGE (CPM)
C               SSYRF- ACTIVITY OF FULL DOSE SYRINGE (CPM)
C               STARE- WEIGHT OF TISSUE (OR ORGAN) PLUS WEIGHING
C                        PAPER
C                TARE- WEIGHT OF WEIGHING PAPER
C                 TAD- TOTAL ADMINISTERED DOSE
C                TAIL- ACTIVITY OF TAIL (CPM)
C               TSCTS- ADJUSTED STANDARD COUNTS
C                  WT- ANIMAL WEIGHT (INPUT)
C*******************************************************************************
C*******************************************************************************
      LOGICAL*1 RADP(3Ø), SPEC(3Ø), DATE(3Ø)
      INTEGER ORN, II(2Ø)
      INTEGER*4 KT, NR
      REAL*8 N(2Ø)
      REAL RAT(2Ø), ACT(5Ø), TARE(5Ø), STARE(5Ø),
     1CALIB(2Ø,2), PPADGS(5Ø), RATIO(5Ø), PMBC(5Ø),
     2TUMOR, WT(4Ø), ASYRF(4Ø), ASYRE(4Ø), TAIL(4Ø),
     3TAD(4Ø), ASYRC(4Ø), SSYRC(4Ø), SSYRF(4Ø), SSYRE(4Ø)
     4,DF(4Ø), BGD(4Ø), SCTSM(4Ø), TSCTS(4Ø), PTADT(4Ø)
      COMMON /A/ N,RAT,CALIB/B/ II,WT,TAD,ACT,TARE,STARE,ORN
      WRITE(5,49)
49    FORMAT(1HØ,'ENTER THE NUMBER OF STANDARDS IN THE PRESENT
     1 RUN'//$' ISTAND=')
      READ(5,55)ISTAND
55    FORMAT(I3)
```

```
      DO 4ØØ IJJ=1,ISTAND
      WRITE(5,1)
1     FORMAT(1HØ,'IF YOU USED THE LIQUID SCINTILLATION COUNTER,
      1 ENTER 1; OTHERWISE, ENTER Ø'//$' IZZZZ=')
      READ(5,4) IZZZZ
4     FORMAT(I2)
      WRITE(5,8)
8     FORMAT(1HØ,'ENTER THE NUMBER OF ANIMALS IN YOUR STUDY,
      1 NR'//$' NR=')
5     READ(5,2ØØ) NR
2ØØ   FORMAT(2I3)
      ORN=16
      WRITE(5,11)
11    FORMAT(1HØ,'ENTER THE SPECIES OF THE ANIMAL IN YOUR
      1 STUDY AND ANY OTHER PERTINENT INFORMATION, USING A MAXIMUM
      2 OF 3Ø SPACES.'//$)
      READ(5,2Ø3)SPEC
2Ø3   FORMAT(3ØA1)
      WRITE(5,13)
13    FORMAT(1HØ,'ENTER THE RADIOISOTOPE USED AND THE COMPOUND
      1(USE A MAXIMUM OF 3Ø SPACES)'//$)
      READ (5,48)RADP
48    FORMAT(3ØA1)
1Ø    WRITE (5,14)
14    FORMAT(1HØ,'ENTER THE FOLLOWING DATA:'/)
      WRITE(5,15)
15    FORMAT(1HØ,'THE NUMBER OF COUNTS PER MINUTE IN THE FULL STANDARD
      1 SYRINGE'//$' SSYRF=')
      READ(5,1ØØ)SSYRF(IJJ)
      WRITE(5,17)
17    FORMAT(1HØ,'THE NUMBER OF COUNTS PER MINUTE IN THE EMPTY STANDARD
      1 SYRINGE'//$' SSYRE=')
      READ(5,1ØØ) SSYRE(IJJ)
      WRITE(5,19)
19    FORMAT(1HØ,'THE DILUTION FACTOR'//$' DF=')
      READ(5,1ØØ) DF(IJJ)
      WRITE(5,21)
21    FORMAT(1HØ,'THE NUMBER OF COUNTS PER MINUTE OF THE DILUTED STANDARD
      1 IN THE AUTOMATIC WELL COUNTER'//$' SCTSM=')
      READ(5,1ØØ) SCTSM(IJJ)
      WRITE(5,23)
23    FORMAT(1HØ,'BACKGROUND FROM THE WELL COUNTER'//$' BGD=')
      READ(5,1ØØ) BGD(IJJ)
      WRITE(5,7Ø)SSYRF(IJJ),SSYRE(IJJ),DF(IJJ),SCTSM(IJJ),BGD(IJJ)
7Ø    FORMAT(1ØX,'SSYRF',3X,F1Ø.Ø,/,1ØX,'SSYRE',3X,F1Ø.Ø,/,1ØX,
      1'DF',6X,F1Ø.Ø,/,1ØX,'SCTSM',3X,F1Ø.Ø,/,1ØX,'BGD',5X,F1Ø.Ø)
      WRITE(5,72)
72    FORMAT(1X,'DO YOU WANT TO CHANGE ANY OF THE ABOVE VALUES?
      1(Ø=NO, 1=YES)'//$' IFCHNG=')
      READ(5,74)IFCHNG
74    FORMAT(I2)
      IF(IFCHNG.GT.Ø) GO TO 1Ø
      SSYRC(IJJ) = SSYRF(IJJ) - SSYRE(IJJ)
      TSCTS(IJJ) = (SCTSM(IJJ) - BGD(IJJ)) * DF(IJJ)
      DO 2 I = 1, NR
24    WRITE(5,25)
25    FORMAT(1HØ,'WEIGHT OF ANIMAL I (GRAMS)'//$' WT(I)=')
      READ(5,1ØØ)WT(I)
      WRITE(5,27)
27    FORMAT(1HØ,'THE NUMBER OF COUNTS PER MINUTE IN THE FULL DOSE SYRINGE
      1 FOR ANIMAL I'//$' ASYRF(I)=')
```

```
      READ(5,1ØØ)ASYRF(I)
      WRITE(5,29)
29    FORMAT(1HØ,'THE NUMBER OF COUNTS PER MINUTE IN THE EMPTY DOSE
      1 SYRINGE FOR ANIMAL I'//$' ASYRE(I)=')
      READ (5,1ØØ) ASYRE(I)
      WRITE(5,31)
31    FORMAT(1HØ,'THE NUMBER OF COUNTS PER MINUTE IN THE TAIL OF ANIMAL I
      1 (IF NO TAIL, ENTER Ø.Ø)'//$' TAIL(I)=')
      READ(5,1ØØ) TAIL(I)
1ØØ   FORMAT(F1Ø.Ø)
      WRITE(5,8Ø)WT(I),ASYRF(I),ASYRE(I),TAIL(I)
8Ø    FORMAT(1ØX,'WT',6X,F1Ø.Ø,/,1ØX,'ASYRF',3X,F1Ø.Ø,/,1ØX,'ASYRE',
      13X,F1Ø.Ø,/,1ØX,'TAIL',4X,F1Ø.Ø)
      WRITE(5,82)
82    FORMAT(3X,'DO YOU WANT TO CHANGE ANY OF THE ABOVE VALUES?
      1(Ø=NO, 1=YES)'//$' IFCHNG=')
      READ(5,84)IFCHNG
84    FORMAT(I2)
      IF(IFCHNG.GT.Ø) GO TO 24
      WRITE(5,33)
33    FORMAT(1HØ,'ENTER THE DISTRIBUTION TIME, STUDY DATE, ANIMAL IDENTI
      1FICATION NUMBER(USE 3Ø SPACES, MAX)'//$)
      READ(5,2Ø4)DATE
2Ø4   FORMAT(3ØA1)
C
C        CALCULATE THE TOTAL ADMINISTERED DOSE
C
      ASYRC(I) = ASYRF(I) - ASYRE(I)
      TAD(I)=((ASYRC(I)/SSYRC(IJJ))*TSCTS(IJJ))-TAIL(I)
C
C        CALCULATE THE PERCENT TAD IN THE TAIL
C
      PTADT(I)=(TAIL(I)*1ØØ.)/(TAD(I)+TAIL(I))
      TUMOR = Ø.Ø
      ZDB = Ø.Ø
      TDB = Ø.Ø
      DO 3 JJ = 1, ORN
      PPADGS(JJ)=Ø.Ø
      PMBC(JJ)=Ø.Ø
3     CONTINUE
      WRITE(5,34)
34    FORMAT(1HØ,'ENTER THE COUNTS PER MINUTE AND WEIGHTS FOR THE FOLLOWING
      1 ORGAN SAMPLES:')
      DO 37 JKL=1,ORN
      WRITE(5,35)JKL,N(JKL)
35    FORMAT(1HØ,I3,1X,A8,2X,'ACT(JKL),TARE(JKL),STARE(JKL)='//$)
      READ(5,36) ACT(JKL), TARE(JKL), STARE(JKL)
36    FORMAT(3F1Ø.Ø)
37    CONTINUE
      WRITE(5,6Ø)
6Ø    FORMAT(1HØ,'DID YOU MAKE A MISTAKE WHILE ENTERING THE
      1ORGAN SAMPLE DATA?(Ø=NO, 1=YES)'$)
      READ(5,61) MSTAKE
61    FORMAT(I2)
      IF(MSTAKE .EQ. 1) GO TO 62
47    IF(IZZZZ .EQ. Ø) GO TO 1Ø5
      CALL CALLIQ
1Ø5   WRITE(4,9)SPEC,WT(I),TAD(I),RADP,DATE
9     FORMAT(1H1,'SPECIES',23X,3ØA1/1H ,'WEIGHT GRAMS',17X,F1Ø.4/1H ,
      1'TOTAL ADMINISTERED DOSE CTS.',2X,F1Ø.Ø/1H ,'RADIOPHARMACEUTICAL
      2',11X,3ØA1/ 1H ,'DISTRIBUTION TIME(HR)',9X,3ØA1,//)
```

```
      IF (IZZZZ .NE. Ø) GO TO 11Ø
      DO 555 K=1, ORN
      IF(TARE(K) .LT. STARE(K)) GO TO 555
      AH = STARE(K)
      STARE(K)=TARE(K)
      TARE(K) =AH
555   CONTINUE
11Ø   WRITE(4,12)
12    FORMAT(1HØ,'ORGAN',8X,'SAMPLE WT.',8X,'ACTIVITY',8X,'TOTAL',8X,
     1'P. ADM. DOSE/',7X,'P. ADM. DOSE/',8X,'P. ADM. DOSE/'/1H ,47X,
     2'ORGAN WT.',4X,'G. SAMPLE',11X,'1% BODY WEIGHT',7X,'G. HUM. WEIGHT
     3')
      DO 6 J = 1, ORN
      KT = ACT(J)
      IF (KT .EQ. Ø) GO TO 51
      WTZ=STARE(J)-TARE(J)
      ORWT=WT(I)*RAT(J)
      PADS=((ACT(J)-BGD(I))/TAD(I))*1ØØ.
      PADGS=PADS/WTZ
      PPADGS(J)=PADGS
      PADPBW=PADGS*WT(I)*Ø.Ø1
      PADGHW=PADPBW/7ØØ.Ø
      PMBC(J)=PADGS*WT(I)
      IF(J.EQ.6)AMUSC=PADGS
      IF(J.EQ.12)BLOOD=PADGS
      IF(J.EQ.16)TUMOR=PADGS
      WRITE(4,7)N(J),WTZ,KT,ORWT,PADGS,PADPBW,PADGHW
7     FORMAT(1HØ,A8,5X,F8.4,8X,I1Ø,8X,F9.4,5X,F11.7,8X,F12.9,9X,F12.9
     1)
      GO TO 6
51    WRITE(4,52)N(J)
52    FORMAT(1HØ,1A6,1ØX,'INSUFFICIENT DATA.....INSUFFICIENT DATA')
6     CONTINUE
      IF(BLOOD .EQ. Ø.Ø) GO TO 3Ø9
      IF(AMUSC .EQ. Ø.Ø) GO TO 3Ø9
      ZDB=TUMOR/BLOOD
      TDB=TUMOR/AMUSC
      WRITE(4,9Ø)ZDB,TDB
9Ø    FORMAT(1HØ,'TUMOR/BLOOD',F9.4,5X,'TUMOR/MUSCLE',F9.4,'(PADGS VALUES)
     1')
3Ø9   IF(TUMOR.EQ.Ø.Ø) GO TO 351
      DO 31Ø L = 1, ORN
      RATIO(L) = Ø.Ø
31Ø   CONTINUE
      DO 3ØØ J = 1, ORN
      IF(PPADGS(J).EQ.Ø.Ø) GO TO 3ØØ
      RATIO(J)=TUMOR/PPADGS(J)
3ØØ   CONTINUE
      WRITE(4,3Ø5)
3Ø5   FORMAT(1H1,'ORGAN',7X,'TUMOR-ORGAN RATIOS',
     17X,'P. MEAN BODY CONC.'/)
      WRITE(4,3Ø6)(N(J),RATIO(J),PMBC(J),J=1,ORN)
3Ø6   FORMAT(1HØ,1A6,7X,F16.8,7X,F16.8)
      GO TO 354
351   WRITE(4,349)
349   FORMAT(1H1,//'ORGAN',12X,'P. MEAN BODY CONC.'/)
      WRITE(4,352)(N(JJ),PMBC(JJ),JJ=1,ORN)
352   FORMAT(1HØ,A8,7X,F16.8)
354   WRITE(4,355)PTADT(I)
355   FORMAT(1HØ,//' PERCENT TAD IN TAIL IS',F8.4)
      WRITE(5,5Ø)(JJJ,N(JJJ),ACT(JJJ),TARE(JJJ),STARE(JJJ),JJJ=1,ORN)
```

```
5Ø      FORMAT(1X,I3,1X,A8,3F12.4)
C
C       OPTION TO CHANGE ANY INPUT DATA AND PRINT NEW RESULTS.
C
62      WRITE(5,38)
38      FORMAT(1HØ,'DO YOU WANT TO CHANGE ANY OF THE VALUES
        1OF ACTIVITY OR WEIGHT?(Ø=NO, 1=YES)'//$' IFCHNG=')
        READ (5,39) IFCHNG
39      FORMAT (I3)
        GO TO 41
45      WRITE(5,4Ø)
4Ø      FORMAT(1HØ,'DO YOU WANT TO CHANGE ANY MORE VALUES
        1 OF ACTIVITY OR WEIGHT?(Ø=NO, 1=YES)'//$' IFCHNG=')
        READ(5,39) IFCHNG
        IF(IFCHNG .LT. 1) GO TO 47
41      IF (IFCHNG .LT. 1) GO TO 2
        WRITE(5,42)
42      FORMAT(1HØ,'ENTER THE NUMBER OF THE ORGAN WHOSE DATA YOU
        1WANT TO CHANGE(1 FOR LUNG, 2 FOR LIVER, ETC.)'//$' ID=')
        READ(5,39) IDENT
        WRITE(5,43) N(IDENT)
43      FORMAT(1HØ,'ENTER THE NEW DATA FOR THE ORGAN:',
        1A8,//$' ACT(ID),TARE(ID),STARE(ID)=')
        READ(5,44) ACT(IDENT),TARE(IDENT),STARE(IDENT)
44      FORMAT(3F1Ø.Ø)
        GO TO 45
2       CONTINUE
4ØØ     CONTINUE
99Ø     WRITE(4,999)
999     FORMAT(1H1,///25X,' END OF RUN ')
        STOP
        END
```

APPENDIX B

ANOVA2 COMPUTER PROGRAM

```
C*************************************************************************
C*************************************************************************
C       THIS PROGRAM IS A STATISTICAL ROUTINE TO COMPARE TWO OR MORE
C       GROUPS OF DATA.  THE ACTUAL TEST IS CALLED ANALYSIS OF
C       VARIANCE TEST OF SIGNIFICANCE (ANOVA) AND TESTS GROUPS OF
C       DATA TO DETERMINE WHETHER OR NOT THERE IS A SIGNIFICANT
C       DIFFERENCE BETWEEN THEM.  THE INPUT IS THE DATA IN BLOCK
C       FORM, WHERE EACH BLOCK REPRESENTS MANY TRIALS OF ONE FACTOR
C       VERSUS ANOTHER (FOR EXAMPLE, TEN DIFFERENT EXPERIMENTS FOR
C       ONE COMPOUND AT ONE TIME, EACH DATUM IS A TUMOR-TO-MUSCLE RATIO
C       FOR THE COMPOUND AT THE GIVEN TIME).  THE DATA MUST BE IN
C       A RECTANGULAR ARRAY, NO SMALLER THAN 2X2 BLOCKS OF DATA.
C       THE BASIC EQUATIONS WERE OBTAINED FROM C. SHERIDAN'S
C       "FUNDAMENTALS OF EXPERIMENTAL PSYCHOLOGY, 2ND ED." AND OTHER
C       REFERENCES ARE "SEQUENTIAL TESTS OF STATISTICAL HYPOTHESES"
C       BY GHOSH AND "STATISTICS FOR MEDICAL AND OTHER BIOLOGICAL
C       STUDENTS" BY BERSTEIN AND WEATHERALL.
C       THE OUTPUT IS A GROUP OF "F" RATIOS FOR THE "FACTORS" AND ALSO
C       t VALUES FROM THE STUDENT'S "t"-TEST BETWEEN EACH BLOCK.
C       THE PROGRAM WAS WRITTEN BY JAMES HENRICKS FOR THE RADIOLOGY
C       COMPUTER VAX.
C                                                       JAH   7/2/79
C*************************************************************************
C*************************************************************************
C       LIST OF VARIABLES
C
C       A(I,J,K).........ARRAY OF DATA
C       B(I,J,K).........ARRAY OF DATA
C              I.........COLUMN VARIABLE
C              J.........ROW VARIABLE
C              K.........NUMBER OF DATA POINTS IN BLOCK
C       SUMXA(I,J).......SUM OF DATA IN A BLOCK
C       SUMX2A(I,J)......SUM OF SQUARES OF DATA IN A BLOCK
C       SUMA(I)..........SUMMATION OF SUMXA FOR A GIVEN COLUMN
C       SUMB(J)..........SUMMATION OF SUMXA FOR A GIVEN ROW
C       SUMA2(X).........SUMMATION OF SUMX2A FOR A GIVEN COLUMN
C       SUMB2(J).........SUMMATION OF SUMX2A FOR A GIVEN ROW
C              M.........TOTAL NUMBER OF BLOCKS
C           MCOL.........NUMBER OF COLUMNS
C           MROW.........NUMBER OF ROWS
C           N(I).........NUMBER OF DATA POINTS IN BLOCK I
C           NTOT.........TOTAL NUMBER OF DATA POINTS
C              I.........INCREMENT FOR COLUMNS
C              J.........INCREMENT FOR ROWS
C              K.........INCREMENT FOR DATA IN A BLOCK
C          NB(J).........NUMBER OF DATA POINTS IN A ROW
C          NA(I).........NUMBER OF DATA POINTS IN A COLUMN
C       SUMTOT...........SUMMATION OF THE SUMA FOR THE COLUMNS
C       SUMTT2...........SUMMATION OF THE SUMA2 FOR THE COLUMNS
C            FA.........."F" RATIO FOR THE A FACTORS (EX: FOR COMPOUNDS)
C            FB.........."F" RATIO FOR THE B FACTORS (TIMES)
C           FAB.........."F" RATIO FOR THE AXB RELATION
C             T.........VALUE OF t FOR TWO GROUPS (STUDENT'S "t" TEST)
C*************************************************************************
C*************************************************************************
      PROGRAM ANOVA
      DIMENSION A(8,8,4Ø),B(8,8,4Ø)
      DIMENSION SUMXA(8,8),SUMX2A(8,8),SUMXB(8,8),SUMX2B(8,8)
      DIMENSION SUMB(8),SUMB2(8),SUMA(8),SUMA2(8),N(64),NA(8),NB(8)
      DIMENSION NNN(8,8)
      LOGICAL*1 NAM(11)
```

```
      LOGICAL*1 HEADNG(6Ø)
      WRITE(5,5)
5     FORMAT(1X,' IF YOU WOULD LIKE TO SEE A SAMPLE OF INPUT, TYPE A'//
     1 ' CONTROL C (CTRL C, BOTH KEYS AT THE SAME TIME).  YOU WILL GET'//
     2 ' A $, THEN TYPE THE FOLLOWING: TYPE CMMNT.;1      THEN HIT'//
     3 ' CARRIAGE RETURN.  AFTER THAT YOU WILL HAVE TO RESTART THE'//
     4 ' ANOVA2 PROGRAM.  (OTHERWISE CONTINUE)'///)
      WRITE(5,6)
6     FORMAT(//' ENTER FILE NAME FOR RESULTS;
     1TO GET RESULTS AT THE END OF THE RUN,'/
     2' TYPE "PRINT FILENAME.DAT;*"'/)
      READ(5,7)NAM
7     FORMAT(11A)
      OPEN(UNIT=2,NAME=NAM,TYPE='NEW',FORM='FORMATTED')
      WRITE(5,8)
8     FORMAT(1X,' ENTER 1 IF YOU WANT ONLY THE "T"-TEST'/
     1' WITHOUT THE ANOVA; OTHERWISE, HIT RETURN'//$' ITTEST=')
      READ(5,35)ITTEST
      WRITE(5,11)
11    FORMAT(1X,' WRITE HEADING'//$' HEADNG:')
      READ(5,12)HEADNG
12    FORMAT(6ØA1)
9     WRITE(5,1Ø)
1Ø    FORMAT(1X,' ENTER THE TOTAL NUMBER OF BLOCKS(ROWS TIMES COLUMNS)'
     1//$' M=')
      READ(5,35)M
      WRITE(5,2Ø)
2Ø    FORMAT(1X,' ENTER THE NUMBER OF COLUMNS'//$' MCOL=')
      READ(5,35)MCOL
      WRITE(5,3Ø)
3Ø    FORMAT(1X,' ENTER THE NUMBER OF ROWS'//$' MROW=')
      READ(5,35)MROW
35    FORMAT(I3)
      WRITE(5,225)M,MCOL,MROW
      WRITE(5,116)
      READ(5,39)ICHANG
39    FORMAT(I2)
      IF(ICHANG.GT.Ø) GO TO 9
41    NTOT=Ø
      DO 12Ø I=1,M
      WRITE(5,4Ø)I
4Ø    FORMAT(1X,' ENTER THE NUMBER OF DATA IN BLOCK NUMBER',
     1I3,//$' N=')
      READ(5,11Ø)N(I)
11Ø   FORMAT(I3)
      NTOT=NTOT+N(I)
12Ø   CONTINUE
      DO 112 I=1,M
      WRITE(5,111)I,N(I)
111   FORMAT(2ØX,' N(',I3,')=',I3)
112   CONTINUE
113   WRITE(5,116)
116   FORMAT(' WOULD YOU LIKE TO CHANGE ONE OF THE ABOVE
     1VALUES?'/' (1=YES,Ø=NO)'//$' CHANGE=')
      READ(5,39)IFCHNG
      IF(IFCHNG.LT.1)GO TO 121
      GO TO 41
121   II=Ø
      DO 19Ø I=1,MCOL
      DO 18Ø J=1,MROW
      II=II+1
```

```
      NN=N(II)
      WRITE(5,122)
122   FORMAT(1X,' ENTER A(I,J,K), THE K-TH DATUM IN COLUMN I AND ROW J'//)
      DO 17Ø K=1,NN
      WRITE(5,126)I,J,K
126   FORMAT($,' A(',I3,',',I3,',',I3,')=')
      READ(5,128)A(I,J,K)
128   FORMAT(F1Ø.5)
17Ø   CONTINUE
      SUMXA(I,J)=Ø.Ø
      SUMX2A(I,J)=Ø.Ø
      SUMXB(I,J)=Ø.Ø
      SUMX2B(I,J)=Ø.Ø
      NB(J)=Ø
      SUMB(J)=Ø.Ø
      SUMB2(J)=Ø.Ø
      WRITE(5,175)II
175   FORMAT(1ØX,' END BLOCK # ',I3)
18Ø   CONTINUE
      SUMA(I)=Ø.Ø
      SUMA2(I)=Ø.Ø
      NA(I)=Ø
19Ø   CONTINUE
      II=Ø
      DO 22Ø I=1,MCOL
      DO 21Ø J=1,MROW
      II=II+1
      NN=N(II)
      DO 2ØØ K=1,NN
      WRITE(5,245)I,J,K,A(I,J,K)
2ØØ   CONTINUE
2Ø1   WRITE(5,2Ø2)
2Ø2   FORMAT(' ENTER THE POSITION OF THE DATUM YOU WANT'/
      1' TO CHANGE, ELSE HIT RETURN',//$' K=')
      READ(5,2Ø4)K
2Ø4   FORMAT(I3)
      IF(K.EQ.Ø)GO TO 21Ø
      WRITE(5,2Ø6)I,J,K
2Ø6   FORMAT(' REENTER A(',I3,',',I3,',',I3,')',//$' A=')
      READ(5,2Ø8)A(I,J,K)
2Ø8   FORMAT(F1Ø.5)
      GO TO 2Ø1
21Ø   CONTINUE
22Ø   CONTINUE
      WRITE(2,222)HEADNG
222   FORMAT(1ØX,6ØA1)
      WRITE(2,225)M,MCOL,MROW
225   FORMAT(////,2ØX,' M=',I3,/,17X,' MCOL=',I3,/,17X,
      1' MROW=',I3,///)
      DO 24Ø I=1,M
      WRITE(2,23Ø)I,N(I)
23Ø   FORMAT(' BLOCK NUMBER',I3,' N=',I3)
24Ø   CONTINUE
      II=Ø
      DO 27Ø I=1,MCOL
      DO 26Ø J=1,MROW
      II=II+1
      NN=N(II)
      DO 25Ø K=1,NN
      WRITE(2,245)I,J,K,A(I,J,K)
245   FORMAT(' A(',I3,',',I3,',',I3,')=',F1Ø.5)
```

```
25Ø     CONTINUE
26Ø     CONTINUE
27Ø     CONTINUE
        I=Ø
        DO 3ØØ II=1,MCOL
        DO 29Ø JJ=1,MROW
        I=I+1
        NN=N(I)
        DO 28Ø KK=1,NN
        SUMXA(II,JJ)=SUMXA(II,JJ)+A(II,JJ,KK)
        SUMX2A(II,JJ)=SUMX2A(II,JJ)+(A(II,JJ,KK)**2)
28Ø     CONTINUE
29Ø     CONTINUE
3ØØ     CONTINUE
        IF(ITTEST.GT.Ø)GO TO 641
        DO 4ØØ II=1,MCOL
        DO 39Ø JJ=1,MROW
        SUMA(II)=SUMA(II)+SUMXA(II,JJ)
        SUMA2(II)=SUMA2(II)+SUMX2A(II,JJ)
39Ø     CONTINUE
4ØØ     CONTINUE
        DO 5ØØ JJ=1,MROW
        DO 49Ø II=1,MCOL
        SUMB(JJ)=SUMB(JJ)+SUMXA(II,JJ)
        SUMB2(JJ)=SUMB2(JJ)+SUMX2A(II,JJ)
49Ø     CONTINUE
5ØØ     CONTINUE
        SUMTOT=Ø.Ø
        SUMTT2=Ø.Ø
        DO 55Ø II=1,MCOL
        SUMTOT=SUMTOT+SUMA(II)
        SUMTT2=SUMTT2+SUMA2(II)
55Ø     CONTINUE
        C=(SUMTOT**2)/FLOAT(NTOT)
        SST=SUMTT2-C
        IJ=Ø
        DO 57Ø II=1,MCOL
        DO 56Ø JJ=1,MROW
        IJ=IJ+1
        NA(II)=NA(II)+N(IJ)
56Ø     CONTINUE
57Ø     CONTINUE
        DO 59Ø JJ=1,MROW
        IJ=JJ
        DO 58Ø II=1,MCOL
        NB(JJ)=NB(JJ)+N(IJ)
        IJ=IJ+MROW
58Ø     CONTINUE
59Ø     CONTINUE
        SSSA=Ø.Ø
        DO 6ØØ II=1,MCOL
        SSSA=SSSA+(SUMA(II)**2)/FLOAT(NA(II))
6ØØ     CONTINUE
        SSA=SSSA-C
        SSSB=Ø.Ø
        DO 61Ø JJ=1,MROW
        SSSB=SSSB+(SUMB(JJ)**2)/FLOAT(NB(JJ))
61Ø     CONTINUE
        SSB=SSSB-C
        SUMNET=Ø.Ø
        K=Ø
```

```
      DO 63Ø I=1,MCOL
      DO 62Ø J=1,MROW
      K=K+1
      SUMNET=SUMNET+(SUMXA(I,J)**2)/FLOAT(N(K))
62Ø   CONTINUE
63Ø   CONTINUE
      SSAXB=SUMNET-C-SSA-SSB
      SSERR=SST-SSA-SSB-SSAXB
      DFA=FLOAT(MCOL)-1.
      DFB=FLOAT(MROW)-1.
      DFAXB=DFA*DFB
      DFSST=FLOAT(NTOT)-1.
      DFERR=DFSST-DFA-DFB-DFAXB
      AMSA=SSA/DFA
      AMSB=SSB/DFB
      AMSAXB=SSAXB/DFAXB
      AMSERR=SSERR/DFERR
      FA=AMSA/AMSERR
      FB=AMSB/AMSERR
      FAB=AMSAXB/AMSERR
      MDFA=MCOL-1
      MDFB=MROW-1
      MDFAB=MDFA*MDFB
      MDFERR=NTOT-MDFA-MDFB-MDFAB
      WRITE(2,64Ø)FA,MDFA,FB,MDFB,FAB,MDFAB,MDFERR
64Ø   FORMAT(1ØX,' FA=',F15.8,5X,' DFA=',I3,//,
      1 1ØX,' FB=',F15.8,5X,' DFB=',I3,//,
      2 1ØX,' FAB=',F15.8,5X,' DFAB=',I3,//,
      3 3ØX,' DFERR=',I3,////)
641   WRITE(2,642)
642   FORMAT(38X,' DF',1ØX,'VALUES OF T'//)
      I=Ø
      DO 65Ø IA=1,MCOL
      DO 645 IB=1,MROW
      NNN(IA,IB)=N(I+IB)
645   CONTINUE
      I=I+MROW
65Ø   CONTINUE
      MMCOL=MCOL-1
      MARK=Ø
      DO 81Ø IJ=1,MMCOL
      DO 8ØØ JJ=1,MROW
      INCR=IJ+1
      DO 79Ø II=INCR,MCOL
      SSQR=(SUMX2A(IJ,JJ)-((SUMXA(IJ,JJ)**2)/FLOAT(NNN(IJ,JJ)))+
      1 SUMX2A(II,JJ)-((SUMXA(II,JJ)**2)/FLOAT(NNN(II,JJ))))/
      2        FLOAT(NNN(IJ,JJ)+NNN(II,JJ)-2)
      SE=SQRT(SSQR*((1./FLOAT(NNN(IJ,JJ)))+(1./FLOAT(NNN(II,JJ)))))
      T=(SUMXA(IJ,JJ)/FLOAT(NNN(IJ,JJ))-SUMXA(II,JJ)/
      1FLOAT(NNN(II,JJ)))/SE
      DF=FLOAT(NNN(IJ,JJ)+NNN(II,JJ)-2)
      MARK=(IJ-1)*MROW+JJ
      IMARK=(II-1)*MROW+JJ
      WRITE(2,89Ø)MARK,IMARK,DF,T
79Ø   CONTINUE
8ØØ   CONTINUE
81Ø   CONTINUE
      MMROW=MROW-1
      DO 93Ø IJ=1,MMROW
      DO 91Ø II=1,MCOL
      INCR=IJ+1
```

```
      DO 900 JJ=INCR,MROW
      SSQR1=(SUMX2A(II,IJ)-((SUMXA(II,IJ)**2)/FLOAT(NNN(II,IJ)))
     1       +SUMX2A(II,JJ)-((SUMXA(II,JJ)**2)/FLOAT(NNN(II,JJ))))/
     2       FLOAT(NNN(II,IJ)+NNN(II,JJ)-2)
      SE=SQRT(SSQR1*((1./FLOAT(NNN(II,IJ)))+
     1       (1./FLOAT(NNN(II,JJ)))))
      T=((SUMXA(II,IJ)/FLOAT(NNN(II,IJ)))-(SUMXA(II,JJ)/FLOAT
     1    (NNN(II,JJ))))/SE
      DF=FLOAT(NNN(II,IJ)+NNN(II,JJ)-2)
      MARK=(II-1)*MROW+IJ
      IMARK=(II-1)*MROW+JJ
      WRITE(2,890)MARK,IMARK,DF,T
890   FORMAT(1X,' BETWEEN BLOCKS',I3,' AND',I3,10X,F7.3,5X,F15.7)
900   CONTINUE
910   CONTINUE
930   CONTINUE
      STOP
      END
```

APPENDIX C

DOSE COMPUTER PROGRAM

```
      PROGRAM DOSE
C     THIS PROGRAM CALCULATES THE EXPECTED DOSE DUE TO AN INJECTED
C   RADIONUCLIDE FROM THE MIRD DATA OF SNYDER,ET.AL.-JNM,AUG.1969,SUP.3
C   BOTH THE S VALUES AND THE ORGAN TO ORGAN DOSE ARE DETERMINED. INPUT
C   INCLUDES INFORMATION ON THE DECAY SCHEME OF THE RADIONUCLIDE,THE
C   PERCENT ADMINISTERED DOSE PER GRAM OF TISSUE FOR ALL ORGANS AND THE
C   CHARACTERISTICS OF DECAY OR CLEARANCE FOR EACH ORGAN. THE ADMINISTER
C   ED DOSE IS ALSO INPUT.
C   ************************************************
C   ************************************************
C        153 SM TEST
C     RADIONUCLIDE CHARACTERISTICS-
C        NG= NUMBER OF GAMMA RAYS ABOVE 1Ø KEV
C        EG(N)= ENERGY OF GAMMA RAY N
C        ED(N)= ENERGY OF GAMMA RAY N PRODUCED PER DECAY,IE.EG(N) TIMES
C               THE YIELD.
C     EB= TOTAL BETA ENERGY DEPOSITED,THIS IS APPROXIMATELY 1/3 OF THE
C         MAXIMUM BETA ENERGY,BUT SHOULD BE OBTAINED FROM THE GRAPHS IN
C         JNM,MARCH 1969,VOL 1Ø,SUP 2,DILLMAN.-THIS IS PER DECAY-
C     EC= TOTAL ENERGY DEPOSITED BY CONVERSION ELECTRONS PER DECAY PLUS
C         ANY GAMMA-RAYS OR X-RAYS UNDER 1Ø KEV AND ANY ENERGY DEPOSITED
C         BY AUGER PROCESSES.
C    HF= HALF LIFE OF RADIONUCLIDE IN HOURS
C   DOSE DATA-
C     TD= DOSE IN MICROCURIES
C     A(J,L)=FRACTION OF DOSE PER GRAM OF TISSUE FOR ORGAN J THAT HAS
C            DECAY ORDER L,EXTRAPOLATED BACK TO TIME OF INJECTION.
C                   - THE WHOLE BODY FRACTION.
C     T(J,L)=HALF LIFE OF DECAY OF ORDER L FOR ORGAN J.
C                          IN HOURS.
C     IN THE ABOVE THE TIME HISTORY OF THE RESIDENCE OF RADIONUCLIDE IN
C     EACH ORGAN IS TREATED AS A SUM OF EXPONENTIAL DECAYS EACH WITH
C     INTERCEPT AT TIME=Ø (INJECTION) OF A AND HALFLIFE T. MORE COMPLICA
C     TED COMBINATIONS OF GROWTH AND DECAY MUST BE ENTERED AS A SEPARATE
C     SUBROUTINE WRITTEN BY THE USER.
C     CALCULATIONAL VARIABLES
C     PHI(I,J,K)= ABSORBED FRACTION IN ORGAN I DUE TO ACTIVITY IN ORGAN
C                 J FOR GAMMA RAY ENERGY K.
C                K         E(KEV)
C                1          1Ø
C                2          15
C                3          2Ø
C                4          3Ø
C                5          5Ø
C                6         1ØØ
C                7         2ØØ
C                8         5ØØ
C                9        1ØØØ
C                1Ø       15ØØ
C                11       2ØØØ
C                12       4ØØØ
C      - THIS DATA IS PROGRAM DATA IN TABULAR FORM
C     P(I,J,N)= ABSORBED FRACTION IN ORGAN I DUE TO  ACTIVITY IN ORGAN J
C               FOR GAMMA RAY N.
C     ORGANS CONSIDERED ARE AS FOLLOWS
C       ORGAN NUMBER=J    ORGAN          MASS=AM(J) GMS
C            1             ADRENALS          15.7
C            2             BLADDER           5Ø9.
C            3             STOMACH           4Ø2.
C            4             SMALL INT.      1,696.
C            5       UPPER LARGE INT.        416.
```

```
C           6       LOWER LARGE INT.          276.
C           7             HEART               6Ø3.
C           8             KIDNEYS             288.
C           9             LIVER             1,833.
C           1Ø            LUNG                999.
C           11      TOTAL MARROW            3,ØØØ.
C           12            PANCREAS             61.
C           13            RIBS              1,Ø41.
C           14            PELVIS              9Ø9.
C           15            SPINE             1,331.
C           16            SKULL             1,271.
C           17      TOTAL SKELETON          1Ø,Ø91.
C           18      TOTAL SKIN              2,593.
C           19            SPLEEN              176
C           2Ø            THYROID              19.9
C           21            UTERUS               66.
C           22      TOTAL TRUNK            43,982.
C           23      TOTAL LEGS             2Ø,776.
C           24      TOTAL HEAD              5,278.
C           25      TOTAL BODY             7Ø,Ø36.
C           26            BRAIN             1,47Ø.
C           27            OVARIES              8.8
C           28            TESTICLES            38.
C      S(I,J)=  S -FOR ORGAN I DUE TO ACTIVITY IN ORGAN J,TABLE FOR NUCLI
C              DE MAY BE OUTPUT IF DESIRED.
C      AM(J)= MASS OF ORGAN J
C      AF(J)= 1.44*AM(J)*D*SUM(A(J,L)*T(J,L))
C                    IN MICROCURIE*HOURS.
C      THE TOTAL DOSE FOR ORGAN I IS THEN-
C        D(I)=SUM(S(I,J)*AF(J))
C        WHERE S(I,J)=2.131*PHI(ORGAN I DUE TO ORGAN J FOR GAMMA RAY N)
C                          *ED(N)/AM(I)
C          NOTE   1.Ø MEV = 2.131 GM*RAD/UCI*HR.
C      RESULT PRINTED IN TABULAR FORM.
       DIMENSION EG(1Ø),ED(1Ø),A(28,4),T(28,4),PHI(28,28,12),P(28,28,1Ø),
      ASI(28),S(28,28),AM(28),AF(28),D(28),AAD(28,28)
         REAL*8 RLST(28)
       DIMENSION EST(12),NN(12)
       DIMENSION EF(3Ø)
         LOGICAL*1 NAM(11)
       DATA EST/Ø.Ø1,Ø.Ø15,Ø.Ø2,Ø.Ø3,Ø.Ø5,Ø.1ØØ,Ø.2ØØ,Ø.5ØØ,
      A1.ØØØØ,1.5ØØØ,2.ØØØØ,4.ØØØØ/
       DATA AM/15.7,5Ø9.Ø,4Ø2.Ø,1696.Ø,416.Ø,276.Ø,6Ø3.Ø,288.
      AØ,1833.Ø,999.Ø,3ØØØ.Ø,61.Ø,1Ø41.Ø,9Ø9.Ø,1331.Ø,1271.Ø,1ØØ91.Ø,2593
      B.Ø,176.Ø,19.9,66.Ø,43982.Ø,2Ø776.Ø,5278.Ø,7ØØ36.Ø,147Ø.Ø,8.8,38.Ø/
       DATA RLST/8HADRENALS ,8HBLADDER  ,8HGI(STOM) ,8HGI(SI)
      A   ,8HGI(ULI)  ,8HGI(LLI)  ,8HHEART    ,8HKIDNEYS  ,8HLIVER    ,8H
      BLUNGS    ,8HMARROW   ,8HPANCREAS ,8HSK.-RIB  ,8HSK.-PELV ,8HSK.-SP
      CIN,8HSK.SKULL,8HSKEL-TOT,8HSKIN      ,8HSPLEEN   ,8HTHYROID  ,8
      DHUTERUS   ,8HTRUNK    ,8HLEGS     ,8HHEAD     ,8HTOT.BODY,8HBRAIN
      E    ,8HOVARIES  ,8HTESTICLE/
C      ENTER A(J,L) THEFRACTION ADMINISTERED DOSE PER GRAM OF TISSUE OF
C       ORGAN J FOR DECAY OF ORDER L HERE AS EITHER ONE OR MORE DATA STAT
C      EMENTS OR A LIST OF EQUALITIES. ALSO SET LMAX THE TOTAL ORDER NO.
C      ENTER ALSO T(J,L) THE DECAY HALFLIFE OF ORDER L FOR ORGAN J.
1        WRITE(5,9)
9        FORMAT(//' ENTER FILE NAME FOR RESULTS'/)
         READ(5,11) NAM
11       FORMAT(11A)
3        OPEN(UNIT=2,NAME=NAM,TYPE='NEW',FORM='FORMATTED')
         WRITE(5,4)
```

```
4       FORMAT(///' ENTER "LMAX", MAXIMUM DECAY ORDER'//$' LMAX=')
        READ(5,5) LMAX
5       FORMAT(I2)
C
C       ENTER TOTAL ADMINISTERED DOSE IN MICROCURIES TD
C
        WRITE(5,57Ø)
57Ø     FORMAT(//' ENTER "TD", TOTAL DOSE IN MICROCURIES'//$' TD=')
        READ(5,13) TD
        WRITE(5,59Ø)
59Ø     FORMAT(//' ENTER "NG", NUMBER OF GAMMA RAYS ABOVE 1Ø KEV'//$' NG=')
        READ(5,5) NG
C
C       ENTER BETA ENERGIES, "EB" THE TOTAL BETA ENERGY DEPOSITED, "EC" THE
C       TOTAL ENERGY DEPOSITED IN CONVERSION ELECTRONS, AUGER PROCESSES AND
C       GAMMA RAYS UNDER 1Ø KEV
C
        WRITE(5,73Ø)
73Ø     FORMAT(//' ENTER "EB", TOTAL BETA ENERGY DEPOSITED IN MEV'//$' EB=')
        READ(5,13) EB
        WRITE(5,74Ø)
74Ø     FORMAT(//' ENTER "EC", ENERGY DUE TO CONVERSION ELECTRONS IN MEV'//
     1$' EC=')
        READ(5,13) EC
        WRITE(5,75Ø)
75Ø     FORMAT(//' ENTER "HF", HALF-LIFE OF RADIONUCLIDE IN HOURS'//$' HF=')
        READ(5,76Ø) HF
76Ø     FORMAT(F1Ø.3)
765     WRITE(5,77Ø) LMAX,TD,NG,EB,EC,HF
77Ø     FORMAT(//////28X,'ITEM'//18X,' 1  LMAX=',I2//18X,' 2  TD=',F8.2,
     1' MICROCURIES'//18X,' 3  NG=',I2//18X,' 4  EB=',F8.5,' MEV'//18X,
     2' 5  EC=',F8.5,' MEV'//18X,' 6  HF=',F1Ø.3,' HOURS'//)
        WRITE(5,772)
772     FORMAT(18X,' TO CORRECT ENTER ITEM NUMBER, ELSE HIT RETURN'/////)
        READ(5,53Ø) ISW
        IF(ISW.EQ.Ø) GOTO 7
        GOTO (775,78Ø,785,79Ø,795,8ØØ),ISW
775     WRITE(5,4)
        READ(5,5) LMAX
        GOTO 765
78Ø     WRITE(5,57Ø)
        READ(5,13) TD
        GOTO 765
785     WRITE(5,59Ø)
        READ(5,5) NG
        GOTO 765
79Ø     WRITE(5,73Ø)
        READ(5,13) EB
        GOTO 765
795     WRITE(5,74Ø)
        READ(5,13) EC
        GOTO 765
8ØØ     WRITE(5,75Ø)
        READ(5,76Ø) HF
        GOTO 765
7       WRITE(5,8)
8       FORMAT(//' ENTER "A(J,L)" THE FRACTION ADMINISTERED DOSE PER GRAM OF
     1 TISSUE OF ORGRAN J FOR DECAY ORDER L')
        DO 56Ø L=1,LMAX
        DO 14 J=1,28
        WRITE(5,12) J,L
```

```
12      FORMAT($' A(',I2,',',I2,')=')
14      READ(5,13) A(J,L)
13      FORMAT(F12.1Ø)
5Ø5     WRITE(5,5Ø3)
5Ø3     FORMAT(/////)
        DO 5ØØ I=1,14
5ØØ     WRITE(5,51Ø) I,L,A(I,L),I+14,L,A(I+14,L)
51Ø     FORMAT(1ØX,' A(',I2,',',I2,')=',E12.6,1ØX,'A(',I2,',',I2,')=',E12.6)
        WRITE(5,52Ø)
52Ø     FORMAT(//,12X,' ENTER "X" COORDINATE FOR CHANGE, ELSE ENTER "Ø"'/)
        READ(5,53Ø) ISW
53Ø     FORMAT(I2)
        IF(ISW.EQ.Ø) GOTO 56Ø
        WRITE(5,54Ø) ISW,L
54Ø     FORMAT($' A(',I2,',',I2,')=')
        READ(5,55Ø) A(ISW,L)
55Ø     FORMAT(F12.1Ø)
        GOTO 5Ø5
56Ø     CONTINUE
        WRITE(5,6ØØ)
6ØØ     FORMAT(//' ENTER "EG(N)", THE ENERGY OF GAMMA RAY N'/)
        DO 61Ø I=1,NG
        WRITE(5,6Ø5) I
6Ø5     FORMAT($' EG(',I2,')=')
61Ø     READ(5,13) EG(I)
        WRITE(5,63Ø)
63Ø     FORMAT(//' ENTER "ED(N)", THE AVERAGE ENERGY/DECAY OF GAMMA RAY N'/)
        DO 64Ø  I=1,NG
        WRITE(5,635) I
635     FORMAT($' ED(',I2,')=')
64Ø     READ(5,13) ED(I)
655     WRITE(5,65Ø)
65Ø     FORMAT(////)
        DO 66Ø I=1,NG
66Ø     WRITE(5,67Ø) I,EG(I),I,ED(I)
67Ø     FORMAT( 1ØX,'EG(',I2,')=',F1Ø.6,1ØX,'ED(',I2,')=',F1Ø.6)
        WRITE(5,68Ø)
68Ø     FORMAT(//1ØX,'ENTER COLUMN AND ROW FOR CHANGE, ELSE HIT RETURN'//)
        READ(5,69Ø) ISWC,ISWR
69Ø     FORMAT(2I2)
        IF(ISWC.EQ.Ø) GOTO 72Ø
        IF(ISWC.EQ.2) GOTO 71Ø
        WRITE(5,6Ø5) ISWR
        READ(5,13) EG(ISWR)
        GOTO 655
71Ø     WRITE(5,635) ISWR
        READ(5,13) ED(ISWR)
        GOTO 655
72Ø     CONTINUE
        WRITE(2,77Ø) LMAX,TD,NG,EB,EC,HF
        DO 81Ø L=1,LMAX
        DO 81Ø I=1,14
81Ø     WRITE(2,51Ø) I,L,A(I,L),I+14,L,A(I+14,L)
        WRITE(2,5Ø3)
        DO 82Ø I=1,NG
82Ø     WRITE(2,67Ø) I,EG(I),I,ED(I)
      DO 15 J=1,28
        SM=Ø.Ø
      DO 1Ø L=1,LMAX
       T(J,L)=HF
      IF(L.GT.1) T(J,L)=Ø.Ø
```

```
      SM=A(J,L)*T(J,L)+SM
   1Ø CONTINUE
      AF(J)=1.44*AM(J)*TD*SM
   15 CONTINUE
C       READ IN PHI(I,J,K) ABSORBED FRACTION IN ORGAN I DUE TO ACTIVITY
C      OF ORGAN J FOR GAMMA RAY OF ENERGY K. DIAGNOSTICS FOR READ ERRORS.
        OPEN(UNIT=1,NAME='DOSE.DAT',TYPE='OLD',FORM='FORMATTED',READONLY)
      DO 5Ø I=1,28
      DO 51 J=1,28
      DO 52 K=1,12
      PHI(I,J,K)=Ø.Ø
   52 CONTINUE
      DO 53 L=1,1Ø
      P(I,J,L)=Ø.Ø
   53 CONTINUE
   51 CONTINUE
   5Ø CONTINUE
   9Ø CONTINUE
      READ(1,1ØØ)NFIX,NSET,NTP,NEF
  1ØØ FORMAT(4(I3,2X))
      IF(NFIX.EQ.999) GO TO 2ØØ
      IF(NFIX.EQ.99) J=NSET
      EF(J)=1.Ø
      IF(NEF.EQ.Ø) GO TO 1Ø1
      EF(J)=Ø.Ø
      GO TO 9Ø
  1Ø1 CONTINUE
      IF(NTP.EQ.2) GO TO 111
      DO 11Ø JJ=1,28
      READ(1,12Ø)I,(PHI(I,J,K),K=1,6)
  12Ø FORMAT(I2,8X,6E1Ø.3)
      DO 64 K=1,6
      IF(PHI(I,J,K).EQ.2.Ø) PHI(I,J,K)=Ø.Ø
   64 CONTINUE
      IF(JJ.NE.I) GO TO 112
      GO TO 11Ø
  112 NW=NW+1
      PRINT 113,I,J,JJ,NW,NTP
  113 FORMAT(1X,5(I3,3X),'READ ERROR')
  11Ø CONTINUE
      GO TO 9Ø
  111 CONTINUE
      DO 115 JJ=1,28
      READ(1,12Ø)I,(PHI(I,J,K),K=7,12)
      DO 65 K=7,12
      IF(PHI(I,J,K).EQ.2.) PHI(I,J,K)=Ø.Ø
   65 CONTINUE
      IF(JJ.NE.I) GO TO 122
      GO TO 115
  122 NV=NV+1
      PRINT 113,I,J,JJ,NV,NTP
  115 CONTINUE
      GO TO 9Ø
  2ØØ CONTINUE
C     FIND K FOR EACH GAMMA RAY
      DO 22Ø N=1,NG
      DO 222 K=1,11
      V=EG(N)*1.ØØØØ
      B=EST(K)
      C=EST(K+1)
      IF(V.GT.B.AND.V.LE.C) NN(N)=K
```

```
  222 CONTINUE
      M=NN(N)
C      FIND P(I,J,N), THE ABSORBED FRACTION IN ORGAN I DUE TO ACTIVITY
C      IN ORGAN J FOR GAMMA RAY N.
      DO 223 I=1,28
      DO 224 J=1,28
      P(I,J,N)=PHI(I,J,M)+(EG(N)-EST(M))*(PHI(I,J,M+1)-PHI(I,J,M))/(EST(
     AM+1)-EST(M))
  224 CONTINUE
  223 CONTINUE
  22Ø CONTINUE
C     CALCULATE S(I,J) FOR ORGAN I DUE TO ACTIVITY IN ORGAN J.
        WRITE(2,235)
  235 FORMAT(1H1,'ORGAN           DOSE(RAD)'/1H )
      DO 25Ø I=1,28
        SI(I)=2.131*(EB+EC)/AM(I)
      DO 251 J=1,28
      S(I,J)=Ø.Ø
      DO 252 N=1,NG
      SHO=2.131*P(I,J,N)*ED(N)*EF(J)/AM(I)
        S(I,J)=SHO+S(I,J)
  252 CONTINUE
  251 CONTINUE
C      CALCULATE DOSE TO ORGAN I FROM ACTIVITY IN ALL ORGANS.
      D(I)=Ø.Ø
      DO 3ØØ J=1,28
      D(I)=S(I,J)*AF(J)+D(I)
  3ØØ CONTINUE
C       CALCULATE DOSE TO ORGAN I FROM ACTIVITY IN I AND ALL OTHER ORGANS.
        D(I)=D(I)+SI(I)*AF(I)
        WRITE(2,35Ø) RLST(I),D(I)
  35Ø FORMAT(1X,A9,3X,F12.6,' RAD.')
      DO 29Ø J=1,28
        IF(I.EQ.J)S(I,J)=S(I,J)+SI(I)
      AAD(I,J)=S(I,J)*AF(J)
  29Ø CONTINUE
  25Ø CONTINUE
        WRITE(2,4ØØ) (RLST(I),I=1,1Ø)
  4ØØ FORMAT(1H1,'ORGAN     ',1Ø(2X,A9))
      DO 4Ø3 J=1,28
        WRITE(2,4Ø1)RLST(J),(AAD(I,J),I=1,1Ø)
  4Ø1 FORMAT(1HØ,A9,1Ø(1X,F1Ø.6))
  4Ø3 CONTINUE
        WRITE(2,4ØØ)(RLST(I),I=11,2Ø)
      DO 4Ø4 J=1,28
        WRITE(2,4Ø1)RLST(J),(AAD(I,J),I=11,2Ø)
  4Ø4 CONTINUE
        WRITE(2,4Ø8)(RLST(I),I=21,28)
      DO 4Ø5 J=1,28
        WRITE(2,4Ø6) RLST(J),(AAD(I,J),I=21,28)
  4Ø6 FORMAT(1HØ,A9, 8(1X,F1Ø.6))
  4Ø5 CONTINUE
  4Ø8 FORMAT(1H1,'ORGAN    ',8(2X,A9))
        WRITE(5,899)
899     FORMAT(18X,' ALL DONE!')
9ØØ     END
```

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