SOME EXPERIENCE WITH ^{57}Co-LABELED BLEOMYCIN AS A TUMOR-SEEKING AGENT

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Bleomycin labeled with ^{57}Co was used as a tumor-localizing agent in 132 patients. In patients with pulmonary tumors the primary localization concentrated radioactivity in 52 of the 54 appropriate cases; out of the 22 clinically known metastases, 19 were visible on the scan; 40 unknown metastases especially in hilus and mediastinum were found by this method and subsequently confirmed. In 22 patients with malignant lymphomas, 18 out of 22 known pathologic lymph glands above the diaphragm were visible on the scan; below the diaphragm the results of scanning in lymph glands and spleen were disappointing, probably because of the disturbing concentration of radioactivity in the kidneys, the bladder, the liver, and sometimes the gut. In 25 patients with various other tumors, 16 out of 22 known localizations above the diaphragm were visible; 2 were uncertain and 4 negative. Below the diaphragm the results were usually negative. In 24 patients with benign lesions, uptake of ^{57}Co-bleomycin was visible on the scintigram in 4 patients with cavitating pulmonary tuberculosis, in 2 with pulmonary infections, in 1 with Caplan lesions of rheumatoid arthritis in the lung, and in 1 with sinusitis ethmoidalis. The significance of these results is discussed.

Since the introduction of radioactive agents into medicine, investigators have been looking for materials that selectively concentrate in malignant tumors. In 1971 Nouel, et al (*1,2*) published the first promising results with ^{57}Co-labeled bleomycin as a tumor-seeking agent. Others were able to verify these results in two small series of patients with various kinds of tumors (*3,4*): in liver metastases using a subtraction technique (*5*), in liver tumors (*6*), and in brain tumors (*7*), all with good results. In patients with malignant melanomas and other epidermal tumors the results were variable or disappointing (*8,9*). The purpose of our investigation was to re-evaluate these findings because of their important clinical implications.

Bleomycin, a group of antibiotics with antineoplastic properties that is derived from Streptomyces verticullus, was first isolated in 1962 (*10*). The material consists of basic sulfur-containing glycopeptides and can be separated by paper chromatography into two fractions, A and B. Further separation by column chromatography gives 19 subfractions with a common structure called bleomycinic acid (Fig. 1, where R = OH). They differ in their terminal amine moiety, R (*11*). More than 100 artificial bleomycins have been produced. Bleomycin preparations chelate copper and are obtained from culture filtrates as copper-containing complexes. Before clinical use, most of the copper is removed from the crude material by treatment with 8-hydroxyquinoline.

Within an hour bleomycin concentrates in normal skin, lung, several kinds of tumors, and probably in the liver and kidneys (*1,11–13*). This selectivity is explained by different concentrations of inactivating enzymes in the various tissues. Blood clearance was investigated in five patients: 20 min after injection 1 liter of whole blood contained 8% (s.d. 4) of the injected radioactivity, after 5 hr 1.9% (s.d. 0.4), and after 18 hr 0.1% (s.d. 0.1). These findings agree with Nouel (*2*) and Grove (*14*).

Cobalt-57-bleomycin is excreted mainly by the kidneys: 54% of the dose is excreted within 6 hr after injection, 82% within the first 24 hr, another 7% from 24 to 48 hr after injection, and 1–2% from 48 to 72 hr (Table 1). Others found about the same excretion pattern of bleomycin as measured by radioactivity (*1,14–17*) or bioassay (*18,19*). During the first 12 hr about 99% of the urinary

Received April 20, 1975; revision accepted July 8, 1975.

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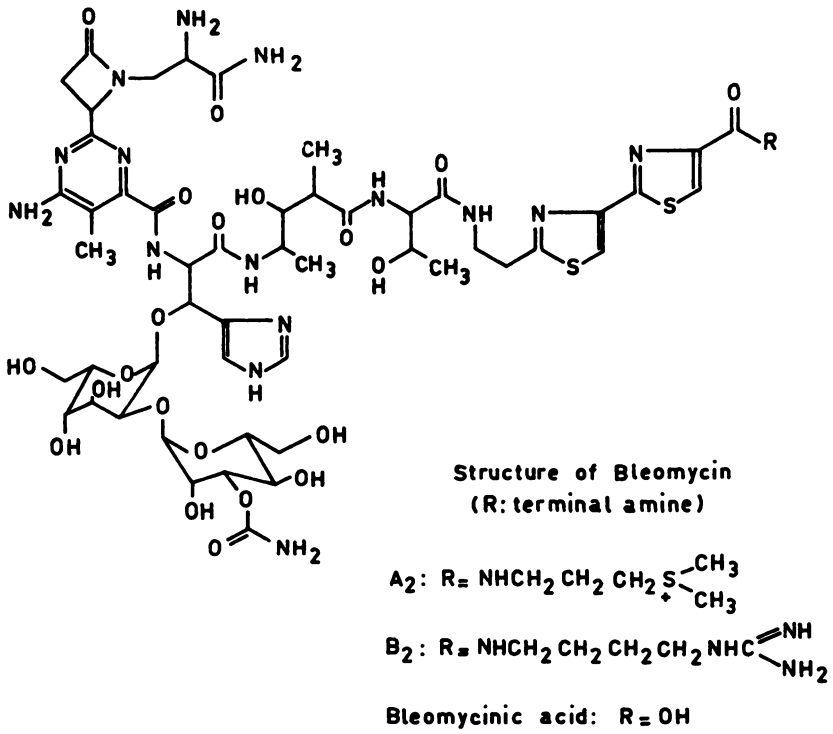

FIG. 1. Structure of bleomycin.

TABLE 1. EXCRETION OF ^{57}Co-BLEOMYCIN IN THE URINE

Time	Number of patients	Percent of injected dose	
		Mean	s.d.
0–6 hr	7	54.4	20.5
0–24 hr	24	81.7	20.6
24–48 hr	9	6.8	4.8
48–72 hr	2	1.5	0.5

radioactivity is ^{57}Co-bleomycin; after this time the percentage of free $^{57}Co^{2+}$ ions in the urine gradually rises until it is about 20% in the urine excreted between 24 and 48 hr (*20*).

As an antineoplastic agent, bleomycin has been used with significant response rates in the treatment of many kinds of tumors (*21–24*), including lymphomas (*25*), epithelial carcinomas of the skin (*26*), the oropharynx, the head and neck, and the lungs.

The tumor-localizing mechanism by ^{57}Co-bleomycin is still unclear. In tumor and liver tissue ^{57}Co-bleomycin is preferentially accumulated in the lysosomes (*27*) and not in the nuclear fraction. Taylor (*28*) found a lysosomal and intranuclear accumulation in tumor cells. From the literature (*29*) it is known that bleomycin attaches itself to DNA, and it is tempting to suppose that this is important in the tumor-seeking mechanism of ^{57}Co-bleomycin.

MATERIALS AND METHODS

Cobalt-57 was obtained as $^{57}CoCl_2$, carrier free, 3 mCi/ml in 0.5 *N* HCl from Philips-Duphar, Petten, The Netherlands. Bleomycin was kindly supplied by Lundbeck N.V., Amsterdam, who obtained it from Nippon Kayaku Co., Tokyo. Batches 72 L 21 and 72 I 05 were used, with a potency of 15.8 and 15.1 mg per vial, respectively, containing the following percentages for the two batches: copper, 0.005–0.003; A_1, 3.9–1.7; B_1, 0.3–0.3; A_2, 64.5–65.8; A'_2, 0.4–0.0; B_2, 29.8–30.4; B_4, 0.0–0.5; A_5, 1.1–1.5. The totals for the B group were 30.1–31.2. The materials were supplied as sterile lyophylized powders in 5-ml ampules.

Labeling procedure [see van de Poll, et al (*30*)]. To 1 ml of $^{57}CoCl_2$ in 0.5 *N* HCl, 7.5 mg bleomycin in 1 ml 0.9% sterile NaCl, 2.5 ml 0.1 *M* acetate buffer (pH 5.6), and, under vigorous stirring, 0.5 ml 1 *N* NaOH are added. The pH of the end product is between 5 and 6. For each patient about 1 mCi ^{57}Co-bleomycin containing about 2.5 mg bleomycin was slowly injected intravenously within a few hours after preparation of the compound.

Radiochemical purity. This is determined by paper electrophoresis (Cellulose acetate, 0.05 *M* acetate buffer, pH 5.6, 32 V/cm, 10 min) and thin-layer chromatography (Kieselgel 60, Alufolie N 20 × 20 cm, Merck); as eluent, ammonia acetate 10% solution in water and methanol 1:1. In this system the R_f of $^{57}CoCl_2$ is 0.

TABLE 2. CONCENTRATION IN NORMAL ORGANS AND TISSUES

Tissues	Frequency	Time	Intensity	Correlation with free $^{57}Co^{2+}$*
Kidneys	Always	First 72 hr	High	—
Bladder	Always	First 24 hr	High	—
Liver	Always	Permanent	Variable	+
Thyroid	35%	First 24 hr	Slight	+
Intestinal tract	Sometimes	First 48 hr	Variable	+
Spleen	Zero			
Bone	Zero			
Salivary glands	Sometimes	First 24 hr	Slight	+
Hypopharynx	Always	First 48 hr	Slight	—
Joints	Sometimes	First 48 hr	Slight	+
Healing wounds	Sometimes	Permanent	Variable	

* + indicates more than 0.5%.

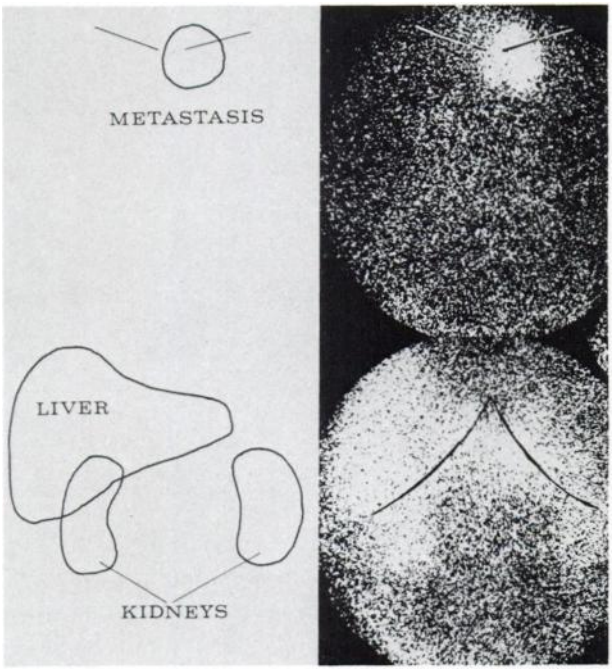

FIG. 2. Patient with metastasis in first lumbar vertebra from anaplastic carcinoma of unknown origin. Anterior scintigrams of thoracic and abdominal regions show radioactivity in normal liver and kidneys and, unexpectedly, in left retroclavicular area. Lumbar metastasis cannot be distinguished on this anterior scintigram; patient was too ill to permit posterior scintigram.

Scintigraphy. A scintillation camera (Searle Radiographics HP) with a parallel-hole collimator (15,000 holes), or with a Divcon diverging collimator, was used. Photographs were taken 24 and/or 48 hr after injection, sometimes even after 72 or 96 hr. A triple-lens Polaroid camera was employed, with 10 min of preset time. When the counts appeared to be less than 10,000, an exposure of 15 or 20 min was used until at least 10,000–15,000 counts were registered. All images were obtained by the same technique, regardless of the area scanned. Data were also stored in a 4,096-channel dual-parameter analyzer for calculating the tumor-to-nontumor (T:NT) ratio. The total number of counts was calculated in a region of interest within the tumor and in one or more regions of equal size around the tumor (usually the regions above and below) or in a corresponding region on the contralateral side of the thorax. From these figures the T:NT ratio was calculated as the ratio of the tumor counts to the mean of the neighboring or contralateral activity.

Interpretation. All scintigrams were interpreted by two of the investigators (JJR and HB). When opinions differed the result was called uncertain; this is noted in the tables where applicable.

All patients were inpatients of the University Hospital, Groningen, mostly in the Department of Internal Medicine (Prof. E. Mandema, M.D.).

THE NORMAL SCINTIGRAM

Concentration in normal organs and tissues (Table 2). In all patients a high concentration of ^{57}Co-bleomycin was found in the kidneys, especially within the first 72 hr, and in the bladder, mainly within 24 hr. In all patients the liver showed concentrations of varying intensity, especially high when there was more than 0.5% free $^{57}Co^{2+}$ in the injected solution (Fig. 2). This seemed to be a quantitative relationship. In the thyroid a slight concentration was found in about 35% of the patients, especially within the first 24 hr and if there was more than 0.5% of free $^{57}Co^{2+}$ in the radiopharmaceutical. In the intestinal tract slight concentration was found, especially in the colon, in about 25% of the investigated patients: this also correlated with the percentage of free $^{57}Co^{2+}$. In the absence of constipation, the intestines were free of radioactivity after 48–72 hr. We have never seen uptake in normal spleen or bones. In the salivary glands we occasionally saw a slight concentration together with scanty uptake in the region of the hypopharynx. In the costosternal cartilages and the humeroscapular joints

FIG. 3. Patient with bronchial carcinoma in left upper lobe and metastasis in brain. (A) Anterior scintigram of thorax shows radioactivity in tumor; (B) anterior scintigram of thorax with lines indicating clavicles; and (C) left lateral view of skull showing radiocobalt activity in brain metastasis (line indicates border of skull).

TABLE 3. GROUP I (PULMONARY TUMORS, 63 PATIENTS)

A Untreated bronchial cancer	
Planocellular carcinoma	30
Oat-cell carcinoma*	6
Not further classified	15
Carcinoma solidum	1
Adenocarcinoma	1
Total	53
B Surgically treated bronchial cancer	
Planocellular carcinoma	5
Not further classified	1
Total	6
C Lung metastases	
Adenocarcinoma of colon	1
Adenocarcinoma solidum	1
Mammary carcinoma	1
Planocellular rhinopharyngeal carcinoma	1
Total	4

* In one of these patients the primary tumor in the lung has never been found.

TABLE 4. GROUP I (PULMONARY TUMORS, 63 PATIENTS)

Localization	Untreated (A)	Surgically treated (B)	Metastases (C)
Primary localization	50*† 2‡	2* ||	
Metastases			
Hilus	13*	3*	3*
Mediastinum	10*	2*	2*
Lung	4* 2§	3*	3* 1§
Supraclavicular	2*	2*	
Brain	4*		
Liver	2*		
Orbit	1*		
Spine	2*		
Elsewhere	1*	2*	

* Definite abnormal focus of activity at site.
† In one of these patients the primary site of an oat-cell carcinoma has never been found.
‡ Uncertain whether uptake is significant.
§ No abnormal focus of activity.
|| Local recurrence.

slight concentration was seen in about 30% of the patients (Fig. 3). This was the case especially when there was more than 0.5% of free ^{57}Co in the injected solution; some other joints were visible in patients with rheumatoid arthritis. A healing wound sometimes concentrated ^{57}Co-bleomycin; this difficulty was avoided by performing our tests before operations such as a mediastinoscopy.

It is very easy to recognize the concentration in kidneys, bladder, and liver; if the concentration in the colon makes interpretation difficult, one may repeat the scanning procedure after 1 or 2 days, if necessary, after purging. The other localizations rarely presented problems in interpretation: the concentration of radioactivity was slight and never exceeded 1.5 times that in the surrounding tissue; this ratio is usually less than 1.25.

TABLE 5. GROUP I (PULMONARY TUMORS, 63 PATIENTS)

Localization	Newly found metastases	Known metastases
Hilus	14	5
Mediastinum	13	1
Lung	3	7 3*
Supraclavicular	2	2
Brain	4	
Liver	1	1
Spine	2	
Groin lymph gland	1	
Femur		1
Orbit		1
Axilla		1
Totals	40	19 3*

* No abnormal focus of activity; all others had definite abnormal focus of activity at site.

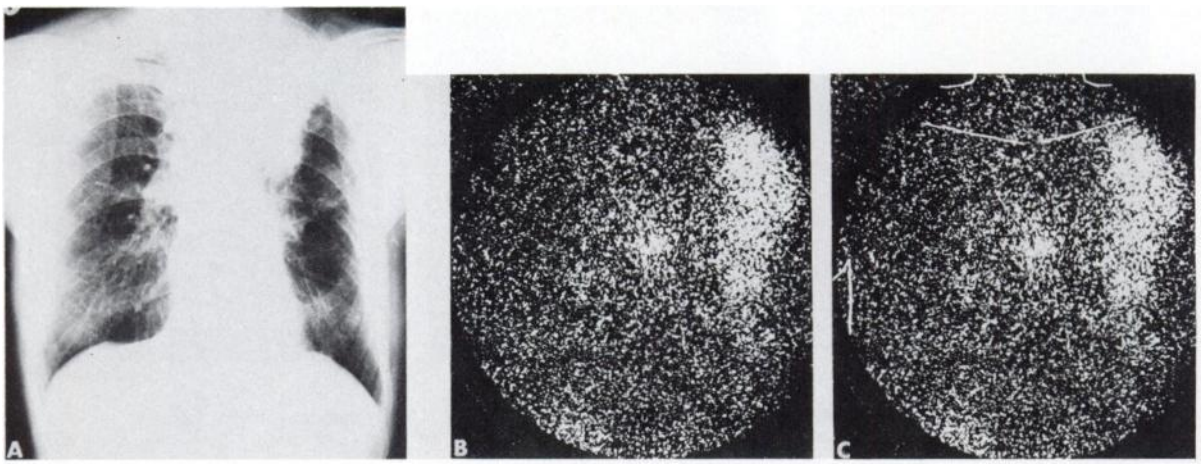

FIG. 4. Patient with planocellular cancer of left lung. (A) Chest x-ray; (B) anterior scintigram of thorax showing radioactivity in tumor and mediastinum (at operation malignant tissue was found in the mediastinum); and (C) same scintigram with lines marking right axilla, clavicles, and outline of neck. Chest x-ray was inconclusive.

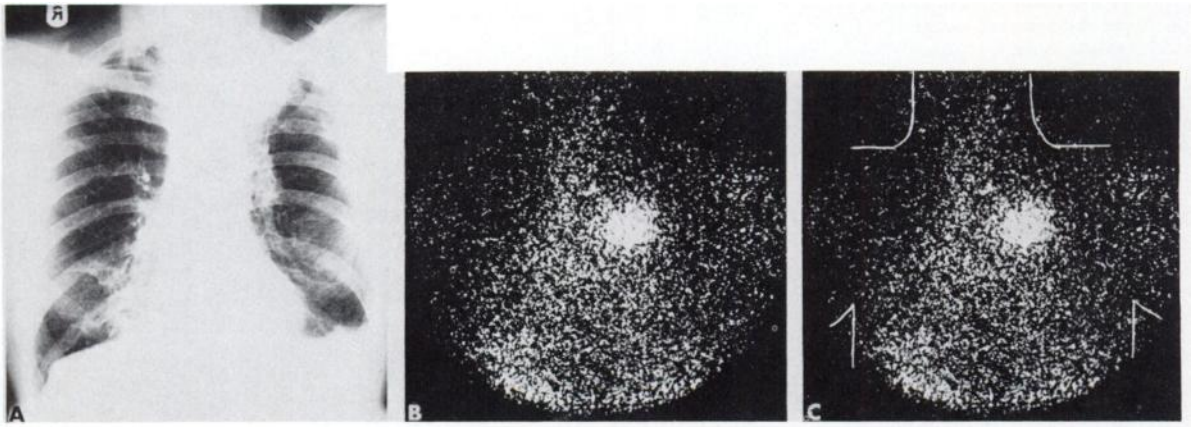

FIG. 5. Patient with old tuberculous scar in left upper lobe of lung. (A) Chest x-ray; (B) anterior scintigram of thorax shows radioactivity in lesion (first indication of malignancy in addition to scar; at operation planocellular cancer was found); and (C) same scintigram with lines marking axillae and neck. Pertechnetate study had been inconclusive.

CLINICAL RESULTS

Based on a preliminary investigation, we divided the 132 patients into four groups for the sake of convenience: Group I, pulmonary tumors (63 patients); Group II, lymphomas (22 patients); Group III, various tumors (25 patients); and Group IV, benign disorders (24 patients).

Group I. This group was divided into three subgroups (Table 3): (A) Untreated patients with bronchial cancer (the primary site for one of the oat-cell carcinomas was unknown, the diagnosis being made on metastases); (B) patients who had been treated surgically for their primary lung cancer and presented one or more metastases or a local recurrence; and (C) patients with metastases in the lung from a primary tumor located elsewhere. (The patient with mammary carcinoma had been operated on and given radiotherapy; the adenocarcinoma of

TABLE 6. GROUP IIA (LYMPHOMAS)

Localization	Hodgkin's disease (11 patients)		Reticulosarcoma (8 patients)		
Above diaphragm					
Neck	2*	3†	4*		
Tonsil			1*		
Supraclavicular			1*		
Axilla	1*	1†	3*		
Mediastinum	5*		1*		
	8*‡	4†	10*‡		
Beneath diaphragm					
Para-aortic		2†		1§	2†
Inguinal			1*	1§	
		2†	1*	2§	2†
Spleen		4†	1*		2† (1 correct)

* Definite abnormal focus of activity at site.
† No abnormal focus of activity.
‡ Of these 18 positive findings 4 were not anticipated.

the colon had been removed surgically 3 years before; lymph nodes of the rhinopharynx cancer had been removed previously and thereafter the neck was treated with radiation.)

The results of Group I are summarized in Table 4. In the scintigrams the primary tumor was clearly distinguishable in 52 of the 54 appropriate cases (96.3%). In two cases only slight concentration was visible; these concerned one small planocellular cancer and one spindle-shaped adenocarcinoma, 1.5 × 0.5 cm.

Of the metastases known at the time of the study with ^{57}Co-bleomycin, 19 showed high concentration of radioactivity whereas 3 did not. These concerned two metastases from a planocellular cancer with diameters of 0.5 and 1 cm, respectively, and one metastasis from an adenocarcinoma of the colon with a diameter of 1.5 cm.

We diagnosed 40 metastases that had not been detected previously by radiologic procedures (tomography, bronchography, etc.). These metastases were located mainly in hilus and mediastinum (Table 5). They were later confirmed by clinical followup studies, mediastinoscopy, thoracotomy, etc. (Figs. 3 and 4).

The T:NT ratio of the primary tumor was estimated in 32 patients, and the average was 2.9 (s.d. 1.2). The primary tumor-to-liver ratio was investigated in 11 patients, and the average was 1.4 (s.d. 0.7).

Several times the ^{57}Co-bleomycin scan provided the first hint that the case involved a malignant tumor of the lung (Fig. 5). In two cases a brain metastasis could be visualized clearly whereas a scintigram with ^{99m}Tc-pertechnetate had been inconclusive.

Group II. This group was subdivided into (A) patients with Hodgkin's disease (11 patients) or reticulosarcoma (8 patients) and (B) patients with chronic leukemia (2 patients) or lymphosarcoma (1 patient).

The results of Group IIA are summarized in Table 6. They appear to differ above and below the diaphragm. As a rule the involved glands above the diaphragm were clearly distinguishable from the background: 18 showed up plainly (4 of these had been unknown until then) and 4 did not. Of the four negative glands, three had almost disappeared clinically after radiotherapy (lymph glands in the neck) and cytostatic treatment (glands in neck and axilla). The T:NT ratio estimated in five patients was 1.8 (s.d. 0.3).

Below the diaphragm the involved glands as a rule were hard to see. Of the locations clinically known, four were indistinguishable from the background, two were dubious, and only one was clearly visible. In the spleen scans, the results were also disappointing. We examined the spleen preoperatively in eight patients: four with Hodgkin's disease and four with reticulosarcoma. The spleen of one patient with reticulosarcoma showed high concentration of radioactivity, and necropsy confirmed our interpretation that the large spleen consisted almost wholly of sarcoma tissue. None of the spleens of the other patients produced visible uptake, but splenectomy showed six of the seven spleens to be neoplastic. In Group II no false-positive results were obtained.

TABLE 7. GROUP III (VARIOUS TUMORS, 25 PATIENTS)

Patients	Abdominal locations	Elsewhere
A Untreated (11)	6†	7* 2†
B Treated (7)	1†	5* 2† 2‡
C Unknown primary tumor (7)	1‡	4*
Total 25 patients	1‡ 7†	16* 4† 2‡

* Definite abnormal focus of activity at site.
† No abnormal focus of activity.
‡ Uncertain whether uptake was significant.

We gave ^{57}Co-bleomycin to five patients with Hodgkin's disease to see if there was local recurrence in the mediastinum after radiotherapy and cytostatic treatment. It is often difficult for the clinician to differentiate between radiation-induced fibrosis and a local recurrence, especially when the site is hilar or mediastinal. In four cases there appeared to be local concentration of ^{57}Co-bleomycin, and local recurrence was later confirmed clinically. In one case there was no concentration of radioactivity, and there is still no evidence of recurrence after more than 6 months.

In Group IIB the involved gland of the patient with lymphosarcoma was surgically removed in toto, the ^{57}Co-bleomycin scan was negative, and in spite of extensive clinical search no other tumor sites could be found. In the two patients with chronic lymphatic leukemia a lymph gland in the neck and one in the mediastinum showed high concentration of radioactivity, but large glands in the axilla and the supraclavicular area were not visible in the scan. The abdominal lymph glands and an inguinal gland also could not be visualized.

Group III. This group was divided into three subgroups: (A) patients (11) with untreated tumors; (B) patients (7) after surgical removal of a primary tumor, with metastases elsewhere (three of these patients had also been irradiated or had received cytostatics for the metastases before the ^{57}Co-

TABLE 8. GROUP IIIA (UNTREATED TUMORS)

Localization	Results of scan: Abnormal focus of activity	Results of scan: No abnormal focus of activity
Beneath diaphragm		
Carcinoma of body of the stomach		1
Plasmocytoma of 5th lumbar vertebra		1
Extraosseous plasmocytoma of perirenal tissue		1
Undifferentiated tumor in duodenum		1
Chordoma in 2nd and 3rd lumbar vertebrae		1
Neuroblastoma of right adrenal gland		1
Elsewhere		
Malignant melanoma of eye		1
Basocellular carcinoma of orbit	1	
Rhabdomyosarcoma of ethmoid bone (Fig. 6)	1	
Planocellular carcinoma of lymph gland metastases	1	1
Nasopharynx carcinoma with skin metastases	3	
Neuroblastoma with cerebral metastases	1	

FIG. 6. Anterior scintigram of skull of 7-month-old child with severe right exophthalmos shows radioactivity in region of right eye. At surgery rhabdomyosarcoma was found. Skull is outlined.

bleomycin investigation); and (c) patients (7) with an unknown primary tumor, the known metastases having been treated by surgery, cytostatics, or radiotherapy.

The results of Group III are summarized in Table 7. In this group also the scans showed great differences between the abdominal region and elsewhere; these were attributed to obscuring uptake in normal organs.

In Subgroup IIIA it appeared impossible to visualize any of the six primary tumors below the diaphragm (Table 8). As a rule neoplasms elsewhere were greatly visible (Fig. 6). Only a malignant melanoma of the eye, smaller than 1 cm in diameter, and one of the metastases from a planocellular cancer of the fossa of Rosenmüller in lymph glands in the neck could not be seen.

In Group IIIB (Table 9) a metastasis from a hypernephroma, in the first lumbar vertebra and thus again below the diaphragm, was not visible on the scan. Tumors elsewhere usually gave good contrast. A metastasis from a breast carcinoma in a lymph gland in the neck, less than 1 cm in diameter, showed only slight concentration (T:NT ratio, 1.5). One of the metastases in the lung from a neuroblastoma was less than 1 cm in diameter and did not show concentration; this patient had been treated with cytostatics before our investigation. The nonconcentrating metastasis in C_4 from the basocellular cancer of the orbit had been treated with radiation. One patient had been operated on for a malignant melanoma of the eye and had no sign of recurrence when we performed the investigation with ^{57}Co-bleomycin; the scan did not show any abnormal uptake.

TABLE 9. GROUP IIIB (PRIMARY TUMOR SURGICALLY REMOVED)

Localization	Results of scan: Abnormal focus of activity	Results of scan: No abnormal focus of activity	Results of scan: Uncertain
Beneath diaphragm			
Metastatic hypernephroma in 1st lumbar vertebra		1	
Elsewhere			
Carcinoma of solidum mammae			
Lung metastases	1		
Lymph gland metastases	1		
Metastatic mammary carcinoma in lymph gland of the neck			1
Basocellular carcinoma of orbit			
Brain metastases	1		
Metastatic in 4th cervical vertebra		1	
Wilms' tumor with lung metastases	1	1	
Hypernephroma with brain metastases	1		
Malignant melanoma of eye (no known metastasis)		1 (correct)	

In seven cases (Group IIIC) we were not successful in locating the primary tumors (Table 10). Even after a followup period of 1–2 years, however, the primary tumor has not been located in any of these patients. In four cases there were no clinical signs of tumor growth at the time of our investigation (presumably as a result of therapy) nor did we find any uptake of ^{57}Co-bleomycin. The remaining three patients had clinically demonstrable metastases. One patient's first lumbar vertebra concentrated ^{57}Co-bleomycin only slightly more than the surrounding tissues and in two cases the remaining metastases showed high concentration even after irradiation (Fig. 2).

Group IV. The patients of Group IV (Table 11) were referred to us to determine whether a clinically discovered abnormality (as a rule in the thorax) was malignant or not. Further clinical study revealed benign disorders. In 16 cases the abnormality did not take up ^{57}Co-bleomycin. In one patient the concentration was slight, and in eight it was definite. In four patients a very active tuberculous cavity was found at thoracotomy; the primary lesions were clearly visible by scintigram, and in three of the four the hilar glands as well. The T:NT ratio in two of the cases was 2.2. One patient with rheumatoid arthritis, silicosis, pulmonary fibrosis, and Caplan lesions in the lungs showed good concentration in the Caplan lesions (T:NT ratio, 2.8). In two patients untreated infection in an atelectatic pulmonary segment accumulated ^{57}Co-bleomycin.

TABLE 10. GROUP IIIC (PRIMARY TUMOR UNKNOWN)

	Results of scan		
	Abnormal focus of activity	No abnormal focus of activity	Uncertain
Clinically no sign of tumor growth			
Carcinoma solidum (treated with cytostatics)		1	
Lymph gland metastases from planocellular carcinoma*		2	
Adenocarcinoma lymph gland metastases*		1	
Clinically apparent metastases			
One undifferentiated carcinoma			
Metastasis in first lumbar vertebra			1
Retroclavicular metastasis	1		
One anaplastic carcinoma solidum with metastasis in humerus	1		
One sarcoma			
Metastasis in femur	1		
Metastasis in lung	1		

* Metastasis surgically removed.

TABLE 11. GROUP IV (BENIGN DISORDERS, 24 PATIENTS)

	Concentration on the scan		
	Abnormal focus of activity	No abnormal focus of activity	Uncertain
Pulmonary embolism		2	
Sarcoidosis		1	
Postpneumonic abnormalities		1	
Lobus venae azygos		1	
Bronchiectasias		3	
Bronchiectasias and atelectasis			1
Old tuberculous scars		2	
Liver abscess		1	
Subphrenic abscess		1	
Abnormal liver scan with ^{99m}Tc-colloid		1	
Exophthalmos of the eye of unknown origin*		3	
Sinusitis ethmoidalis	1		
Cavitating lung tuberculosis	4		
Caplan lesions	1		
Untreated infection in atelectatic pulmonary segment	2		

* One of these patients had the sinusitis ethmoidalis mentioned here.

TABLE 12. RADIATION DOSE OF 1 mCi ^{57}Co-BLEOMYCIN

Organ	Uptake	Time*	Radiation dose (rads)
Liver	5%	$T_{1/2} = 5$ days	0.4
Kidneys	100%	$T_{1/2} = 5$ hr	2.0
Bladder	50%	$T = 3$ hr	1.4
	30%	$T = 9$ hr	
	15%	$T = 12$ hr	
	5%	$T = 2.5$ days	
Total body	80%	$T_{1/2} = 5$ hr	0.09
	20%	$T_{1/2} = 5$ days	

* Given exponential decay the radiation time T equals 1.44 times the biologic half-life, $T_{1/2}$.

GENERAL REMARKS

Tumor size. The smallest tumor detected was a metastasis from a breast cancer: a lymph node in the axilla 0.5 cm in diameter. In the thoracic region our ^{57}Co-bleomycin technique visualized all clinically known and many unrecognized tumors with diameters of more than 1.5 cm.

Adverse reactions. It is known that in 10–50% of the patients, especially in those with lymphomas, a febrile reaction with shock or even death may follow the first injection of bleomycin in therapeutic doses. In one patient with a reticulosarcoma we saw a shaking chill, fever, and shock several hours after the injection of ^{57}Co-bleomycin, and blood cultures were positive for bacteria. Apart from this our patients showed no fever, nausea, vomiting, or anorexia. One patient with a planocellular cancer in the lung complained of pain in the tumor region the night after the ^{57}Co-bleomycin injection. Such reactions after bleomycin are also described in the literature (*24*), and this was actually one of the reasons why people started to evaluate bleomycin as an agent for tumor localization (*31*).

Radiation dose. The maximum whole-body dose for 1 mCi ^{57}Co-bleomycin was calculated to be 0.09 rads (Table 12). Nouel (*1*) calculated 0.5 rads and Grove (*14*) 0.03 rads. The critical organ is the bladder wall, which receives a maximum dose of 1.4 rads [Grove (*14*) calculated 0.45 rads]. The maximum radiation dose to the liver was 0.4 rads and to the kidneys 2.0 rads.

Our estimates were based on the MIRD Tables (*32,33*) and our experimental findings concerning the distribution and excretion of the radiopharmaceutical.

DISCUSSION

In the thoracic region, especially in the lung hili and mediastinum, ^{57}Co-bleomycin seems to be a useful tumor-localizing agent, probably superior to all others known. Almost all primary lung tumors concentrated ^{57}Co-bleomycin clearly and many tumors and metastases in the mediastinum and hili were also well demonstrated. Many of these were not detectable with conventional radiologic methods. The smallest tumor found in the thorax by this method was about 1 cm in diameter; here ^{57}Co-bleomycin scintigraphy should be highly reliable if the tumor is more than 1.5 cm in diameter. In five patients, active tuberculosis and the hilar glands concentrated radioactivity, as did the Caplan lesions of rheumatoid arthritis and untreated pulmonary infection in two patients. No other benign lesions in the lung concentrated ^{57}Co-bleomycin. These findings are in accordance with Nouel (*2*) and Grove (*14*), who mentioned that active inflammation may concentrate the material.

Cobalt-57-bleomycin shows good concentration in lymphomas located above the diaphragm. The scan may be of some help in patients who have received radiotherapy to the mediastinum and in whom local recurrence is difficult to establish. Below the diaphragm the method is handicapped by the variable uptake in liver, kidneys, bladder, and less often in the colon. Because of this, it is often impossible to locate lymphomas or other tumors in the abdominal region. After radiotherapy or cytostatic treatment, the tumors frequently do not concentrate ^{57}Co-bleomycin. Searching for unknown tumors not detectable by extensive clinical investigation was unsuccessful in our series possibly due to small tumor diameter.

In primary and metastatic tumors of the brain the ^{57}Co-bleomycin scan sometimes supplements pertechnetate brain scans (*7*). In our series all seven clinically proven cerebral metastases were clearly visible on the scintigrams. We did not use subtraction techniques for the liver as other authors did (*5*)

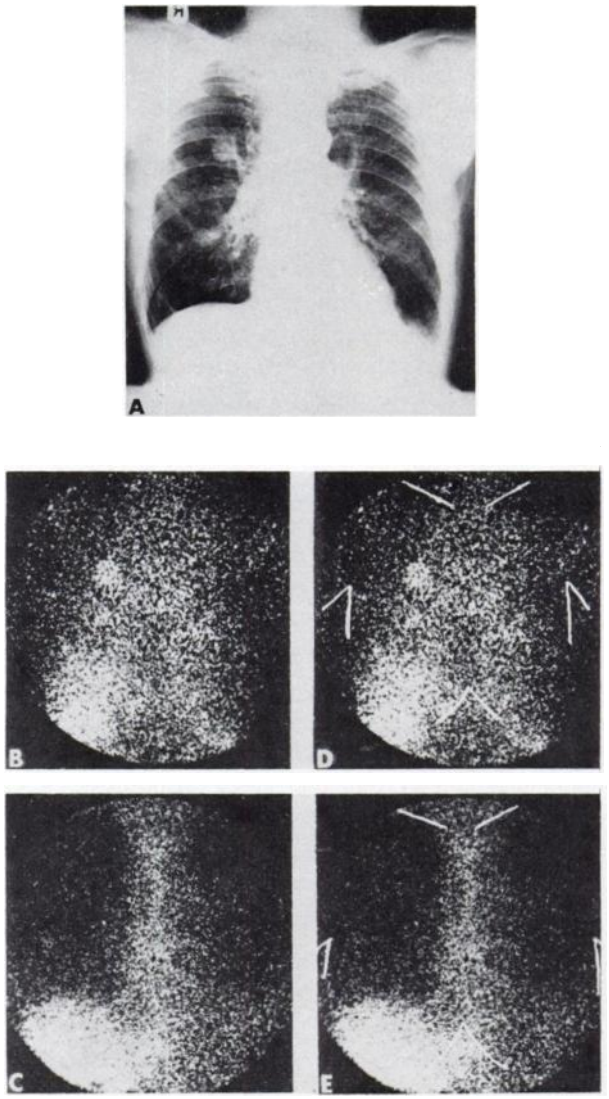

FIG. 7. Patient with planocellular cancer in right upper lobe of lung. (A) Chest x-ray; (B) anterior scintigram of thorax taken with ^{57}Co-bleomycin shows concentration in tumor; (C) similar scan taken with ^{111}In-bleomycin 72 hr after injection shows no concentration in tumor. (D and E) Same scintigrams with lines marking axillae, clavicles, and xiphoid process.

and we did not investigate epidermal tumors since the scan data would add nothing useful.

Outside the abdominal region, the concentration in normal tissues seldom impedes interpretation of the scans. The accumulation of ^{57}Co-bleomycin we found in normal tissues agrees well with Nouel (*2*), who found the same correlation with free $^{57}Co^{2+}$ ions and uptake in liver, colon, and various other tissues (personal communication, 1973). Haubold (*3,5*) and Laconi (*9*) also found uptake of ^{57}Co-bleomycin in normal liver. It is not clear why Grove (*14*) found no concentration in normal liver or colon in patients since he too mentions accumulation in the livers of animals (*15*). This was also found in tumor-bearing animals by Nouel (personal communication, 1973) and Rasker (*20*).

A disturbing artifact may be produced in the scintigram by urinary contamination. In one patient with impaired renal function, a considerable amount of ^{57}Co-bleomycin was present in the circulation even after 72 hr, and an extremely high concentration was found in the colon. This has led us to consider renal impairment an absolute contraindication for ^{57}Co-bleomycin study.

Cobalt-57 has a long physical half-life (270 days); contamination and waste disposal may therefore present a problem. In order to minimize environmental pollution we collected urine during the first 24 hr after injection, thus catching about 80% of the injected dose. Only 7% and 1.5% of the dose was excreted on the second and third day, respectively; further collection of urine, therefore, seems unrewarding.

As have other investigators, our group has been looking for more appropriate radioactive labels for bleomycin, but the results have been disappointing.

Indium-111-bleomycin circulates for a considerable time, thus causing prolonged concentration in the cardiac pool, it gives a high concentration in bones (*14,34–42*), and it probably is unstable in mammals (*28,33,34,43*). In agreement with others, we found ^{57}Co-bleomycin superior to ^{111}In-bleomycin as a tumor-seeking agent in animals (*13,28,38*) and in man (*14,44*) (Fig. 7).

Results with ^{99m}Tc-bleomycin seemed promising (*16,45,46*), but we found prolonged concentration in the blood and inadequate tumor-localizing capability in patients and tumor-bearing animals (*44*).

With ^{67}Cu-bleomycin our results in tumor-bearing animals were disappointing and inferior to those with ^{57}Co-bleomycin. The results in the literature are variable (*2,17,47–49*).

Mercury-197-bleomycin also remains in the circulation for a long time and the complex is probably unstable in vivo (*44*).

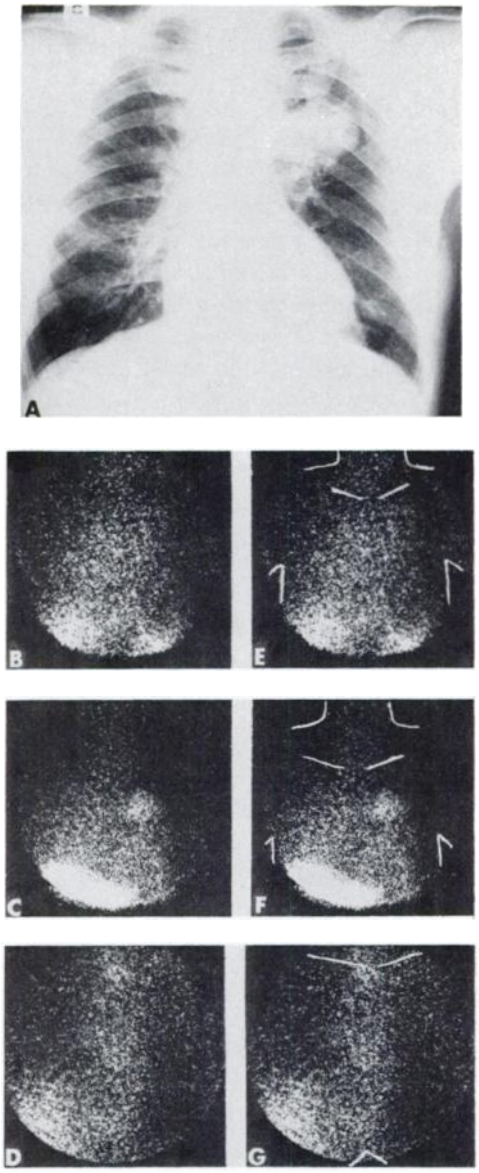

FIG. 8. Patient with anaplastic carcinoma of left lung. (A) Chest x-ray; (B) anterior scintigram of thorax with ^{99m}Tc-bleomycin 24 hr after injection (diverging collimator) shows no concentration in tumor; (C) similar scan taken with ^{57}Co-bleomycin clearly shows tumor; and (D) repeat scan with ^{111}In-bleomycin 72 hr after injection (parallel-hole collimator) shows no concentration in tumor. (E, F, and G) Duplicate photographs with anatomic markings as before.

For these reasons tumors in the hili or mediastinum cannot be visualized well with ^{111}In-, ^{99m}Tc-, and ^{197}Hg-bleomycin. Since these are the only thoracic regions actually giving diagnostic problems to the clinician, investigation with these materials seems unpromising for the staging of lung tumors (Fig. 8).

In animals ^{57}Co-bleomycin gave a better T:NT ratio than ^{67}Ga-citrate (*15*). A comparative study of ^{57}Co-bleomycin and ^{67}Ga-citrate as tumor-seeking radiopharmaceuticals in man has recently been done (*14*): in 15 patients ^{57}Co-bleomycin gave 73%

positive findings whereas ^{67}Ga-citrate scans were positive in only 47%; of 3 squamous-bronchial carcinomas, all were negative with ^{67}Ga-citrate whereas with ^{57}Co-bleomycin 2 were positive and 1 negative. From this study we may not draw final conclusions, but ^{67}Ga concentrates in bones and other tissues besides tumors and shows prolonged concentration in the blood. For these reasons we may suppose that exploration with ^{57}Co-bleomycin is superior to that with ^{67}Ga-citrate in localizing tumors in mediastinum or lung hili.

The long physical half-life of ^{57}Co makes the investigation with ^{57}Co-bleomycin unsuitable for use in a routine program. In selected cases it may give the clinician important new information he will not be able to get with other nonsurgical methods. Until another radioactive label with the same qualities as ^{57}Co has been found, the investigation with ^{57}Co-bleomycin may be indispensable for clinical use.

ACKNOWLEDGMENTS

This study was made possible by a grant from the Koningin Wilhelmina Fonds, Nederlandse Organisatie voor de Kankerbestrijding.

The authors are grateful to J. J. Pratt, C. F. v.d. Merwe, and A. Versluis for their helpful suggestions.

REFERENCES

1. NOUEL JP, RENAULT H, ROBERT J, et al: La bléomycine marquée au Co 57. Intérêt dans le diagnostic des tumeurs malignes et de leur extension. *Nouv Presse Med* 0: 25–28, 1971

2. NOUEL JP, ROBERT J, WITZ H, et al: Le diagnostic des tumeurs malignes et de leur extension par la bléomycine marquée au ^{57}Co. *Radioaktive Isot Klin Forschung* 10: 504–512, 1972

3. HAUBOLD U, SCHMILOWSKI M, JANSSEN B: Erfahrungen mit ^{57}Co-markierten Bleomycin zur Tumorszintigraphie. Presented at the 11th Annual Meeting of the Society of Nuclear Medicine, Athens, September 25–29, 1973

4. JUNG H, GAMM H, FISCHER J, et al: Tumorszintigraphie mit ^{57}Co-Bleomycin. Presented at the 11th Annual Meeting of the Society of Nuclear Medicine, Athens, September 25–29, 1973

5. HAUBOLD U: Kombinierte Lebermetastasendiagnostik mit ^{57}Co-Bleomycin und ^{99m}Tc-Schwefelkolloid. Presented at the 11th Annual Meeting of the Society of Nuclear Medicine, Athens, September 25–29, 1973

6. MAEDA T, TANAKA M: Uptake of ^{57}Co-bleomycin by liver tumor. Tumor scanning with ^{57}Co-bleomycin. *Radioisotopes* 22: 311–313, 1973

7. MAMO L, HOUDART R, REY A: Intérêt de l'exploration à la bléomycine marquée dans le détection des processus expansifs malins intracraniens. *Rev Neurol* 120: 577–582, 1972

8. ROBERT J, NOUEL JP, RENAULT H, et al: Le diagnostic topographique des naevocarcinomes et de leurs métastases à l'aide de la bléomycine marquée. *Bull Soc Franc Derm Syph* 81: 166–171, 1972

9. MORET R, ORTONNE JP, PERROT H, et al: Exploration à la bléomycine marquée en dermatologie. Sa valeur et ses limites dans le diagnostic des épithéliomas cutanés des mélanomes malins et de leurs métastases. *Nouv Presse Med* 3: 193–195, 1974

10. UMEZAWA H, MAEDA K, TAKEUCHI T, et al: New antibiotics, Bleomycin A and B. *J Antibiot Ser A* 19: 200–209, 1966

11. UMEZAWA H: Natural and artificial bleomycin: chemistry and antitumour activities. *Pure Appl Chem* 28: 665–680, 1970

12. ICHIKAWA T, UMEZAWA H, OHASHI O, et al: Animal experiments confirming the specific effect of bleomycin against squamous cell carcinoma. In *Progress in Antimicrobial and Anticancer Chemotherapy, Proceedings of the 6th International Congress of Chemotherapy*, vol 2, Tokyo, University of Tokyo Press, 1970, pp 315–316

13. GROVE RB, ECKELMAN WC, REBA RC: Distribution of labeled bleomycin in normal and tumor-bearing mice. *J Nucl Med* 14: 917–919, 1973

14. GROVE RB, REBA RC, ECKELMAN WC, et al: Clinical evaluation of radiolabeled bleomycin (bleo) for tumor detection. *J Nucl Med* 15: 386–390, 1974

15. KONO A, KOJIMA M: The tumor specific localizing agents for radioisotope image. The preparation of labeled bleomycin and their distributions in the tumor-bearing mice. *Radioisotopes* 21: 118–120, 1972

16. LIN SM, GOODWIN DA: Method of preparation and pharmodynamics of a ^{99m}Tc-bleomycin. *J Nucl Med* 14: 422, 1973

17. LILIEN DL, JONES SE, O'MARA RE, et al: A clinical evaluation of indium-bleomycin as a tumor-imaging agent. *J Nucl Med* 15: 512, 1974

18. FUJITA H, KIMURA K: Blood level, tissue distribution excretion and inactivation of bleomycin. In *Progress in Antimicrobial and Anticancer Chemotherapy, Proceedings of the 6th International Congress of Chemotherapy*, vol 2, Tokyo, University of Tokyo Press, 1969, pp 309–314

19. OHNUMA T, HOLLAND JF, MASUDA H, et al: Microbiological assay of bleomycin: inactivation, tissue distribution and clearance. *Cancer* 33: 1230–1238, 1974

20. RASKER JJ: Opsporing van tumoren met radioactief gemerkt bleomycine. Thesis, Drukkerij van Denderen Groningen, January 1975

21. BLUM RH, CARTER SK, AGRE K: A clinical review of bleomycin, a new antineoplastic agent. *Cancer* 31: 903–913, 1973

22. YAGODA A, MUKHERJI B, YOUNG C, et al: Bleomycin, an antitumor antibiotic. *Ann Intern Med* 77: 861–870, 1972

23. EORTC (European Organization for Research on the Treatment of Cancer): Study of the clinical efficiency of bleomycin in human cancer. *Br Med J* 2: 643–645, 1970

24. BONADONNA G, DE LENA M, MONFARDINI S, et al: Clinical trials with bleomycin in lymphomas and in solid tumors. *Eur J Cancer* 8: 205–215, 1972

25. RUDDERS RA: Treatment of advanced malignant lymphomas with bleomycin. *Blood* 40: 317–331, 1972

26. ICHIKAWA T: The clinical effect of bleomycin against squamous cell carcinoma and further developments. In *Progress in Antimicrobial and Cancer Chemotherapy, Proceedings of the 6th International Congress of Chemotherapy*, vol 2, Tokyo, University of Tokyo Press, 1969, pp 1–3

27. KONINGS AWT, RASKER JJ: Posthuma trumpie: The subcellular distribution of ^{57}Co-bleo and $^{57}CoCl_2$ in tumor and liver of tumor-bearing animals: to be published

28. TAYLOR DM: Comparison of ^{57}Co, ^{62}Zn and ^{111}In bleomycin complexes for tumor localization. In *Radiophar-*

maceuticals, Subramanian G, Rhodes BA, Cooper JF, Sodd VJ, eds, New York, Society of Nuclear Medicine: to be published

29. SUZUKI H, NAGAI K, AKUTSU E, et al: On the mechanism of action of bleomycin strand scission of DNA caused by bleomycin and its binding to DNA in vitro. *J Antibiot* 23: 473–480, 1970

30. V.D.POLL MAPC, RASKER JJ, WOLDRING MG, et al: Labeling of bleomycin: in preparation

31. RENAULT H, RAPIN J, RUDLER M, et al: La chelation de divers cations radioactifs par certains polypeptides utilisée comme méthode de marquage. Application à la bléomycine. *Bull Chimie Thérapeut* 3: 232–235, 1972

32. DILLMAN LT: Radionuclide decay schemes and nuclear parameters for use in radiation dose estimation. MIRD Pamphlet No. 4, *J Nucl Med* 10: Suppl 2, 1966

33. SNYDER WS, FORD MR, WARNER GG, et al: Estimates of absorbed fractions for monoenergetic photon sources uniformly distributed in various organs of a heterogeneous phantom. MIRD Pamphlet No. 5, *J Nucl Med* 10: Suppl 3, 1966

34. MERRICK MV, GUNASEKERA SW, LAVENDER JP, et al: The use of indium-111 for tumour localisation, a comparison of several chelates with gallium-67 in inflammatory and neoplastic lesions, with a note on indium-labeled bleomycin. In *Medical Radioisotope Scintigraphy*, vol 2, Vienna, IAEA, 1972, pp 721–729

35. MERRICK MV, LAVENDER JP, POOLE CW, et al: ^{111}In-labeled bleomycin: clinical experience as a diagnostic agent in tumours of the thorax and abdomen. *Br J Radiol*: to be published

36. THAKUR ML, MERRICK MV, GUNASEKERA SW: Some pharmacological aspects of a new radiopharmaceutical, indium-111-bleomycin. In *Radiopharmaceuticals and Labelled Compounds*, Vienna, IAEA, 1973, pp 183–193

37. RASMUSSEN JW, FREDERIKSEN PB, KALNAES O: ^{111}In-bleomycin compared with ^{67}Ga and ^{75}Se in tumor scintigraphy. Presented at the 11th Annual Meeting of the Society of Nuclear Medicine, Athens, September 25–29, 1973

38. GOODWIN DA, LIN MS, DIAMANTI CI, et al: ^{111}In-labeled bleomycin for tumor localization by scintiscanning. *J Nucl Med* 14: 401, 1973

39. REBA RC: Evaluation of radiolabeled bleomycin for tumor detection by radioisotope scanning. In *Radiopharmaceuticals*, Subramanian G, Rhodes BA, Cooper JF, Sodd VJ, eds, New York, Society of Nuclear Medicine: to be published

40. VERMA R: Relative tissue concentration and radiation dosimetry of ^{111}In-bleomycin. In *Radiopharmaceuticals*, Subramanian G, Rhodes BA, Cooper JF, Sodd VJ, eds, New York, Society of Nuclear Medicine: to be published

41. VERMA RT, TOUYA JJ, BENNETT LR: Further observations on indium-111-labeled bleomycin for scanning tumors in man. *J Nucl Med* 15: 541, 1974

42. YEH SDJ, GRANDO RE, YOUNG CW, et al: Metabolic and scintigraphic studies of ^{111}Indium-Bleomycin in man and tumor-bearing animals. *J Nucl Med* 15: 546, 1974

43. ROBBINS PJ, SILBERSTEIN EB, FORTMAN DL: ^{111}In-bleomycin kinetics in mice bearing transplantable tumors of lung, skin, and bone. *J Nucl Med* 15: 273–278, 1974

44. RASKER JJ, V.D.POLL MAPC, VERSLUIS A, et al: Clinical evaluation of radiolabeled bleomycin for tumor detection: to be published

45. KATO S, KURATA K, IKEDA I, et al: A simple device for preparation of various ^{99m}Tc compounds in sterilized form by an electrolytic method. *J Nucl Med* 14: 415, 1973

46. MORI T, HAMAMATO K, TORIZUKA K: Studies on the usefulness of ^{99m}Tc-labeled bleomycin for tumor imaging. *J Nucl Med* 14: 431, 1973

47. ECKELMAN WC, REBA RC, KUBOTA H, et al: Early detection of mammary carcinoma with radiolabeled bleomycin. *J Nucl Med* 15: 489, 1974

48. COATES G, ASPIN N, WONG PY, et al: ^{67}Cu as a label for bleomycin. *J Nucl Med* 15: 484, 1974

49. HALL JN, O'MARA RE, CRUZ P: ^{67}Cu bleomycin: A tumor-localizing agent. *J Nucl Med* 15: 498, 1974

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Gallbladder Visualization in Adrenal Scanning (Case Report). Accepted 8/6/75.
John C. Harbert, John J. Canary, and Kenneth L. Sandock

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Ronald J. Callahan, Kenneth A. McKusick, Frank P. Castronovo, Myles Lamson, III, and Majic S. Potsaid

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U. Yun Ryo, Ali A. Mohammadzadeh, Aslam Siddiqui, Lelio G. Colombetti, and Steven M. Pinsky

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Ralph S. Wolfstein, Doina E. Tanasescu, Alan D. Waxman, and Jan K. Siemsen

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