[Chem. Pharm. Bull. 31(9)3242—3247(1983)]

Evaluation of ^{99m}Tc-Labeled Amino Acids as Radiopharmaceuticals. V.[1] ^{99m}Tc Complex of Ethylenediamine-*N*,*N*-diacetic Acid as a Scintigraphic Agent for Tumors

YOSHIHARU KARUBE,*,[a] TAKESHI IMOTO,[a] TATSUO MAEDA,[a] MASATO OHYA,[a] SETSURO SUGATA,[a] AKIRA KONO,[a] HARUKAZU MASHIBA,[a] YUKITO ICHINOSE,[a] KOICHI TANAKA,[a] MARIKO KAKU,[a] and YOSHIKAZU MATSUSHIMA[b]

Kyushu Cancer Center Research Institute,[a] Notame, Minami-ku, Fukuoka 815, Japan and Kyoritsu College of Pharmacy,[b] Shibakoen 1–5–30, Minato-ku, Tokyo 105, Japan

(Received February 21, 1983)

Tumor tissues were clearly visualized in scintigrams of experimental animals bearing tumors a few hours after the administration of ^{99m}Tc complex of ethylenediamine-*N*,*N*-diacetic acid. The animals used were mice bearing Ehrlich tumor, mice bearing Sarcoma 180, golden hamsters bearing lymphoma, mice bearing fibrosarcoma induced by 3-methylcholanthrene (MC), rats bearing MC-induced fibrosarcoma that had been transplanted at the limb and had spontaneously metastasized to the lung, and mice bearing spontaneous mammary carcinoma. In the mice bearing Ehrlich tumor, the ratios of radioactivity, tumor/blood and tumor/muscle, were 3.32 and 7.96, respectively, at 3 h after the administration.

Keywords——radiopharmaceutical; tumor imaging; scintigraphy; ^{99m}Tc; ethylenediamine-*N*,*N*-diacetic acid; Ehrlich tumor; Sarcoma 180; lymphoma; fibrosarcoma; mammary carcioma

In the course of studies on ^{99m}Tc labeled amino acids to evaluate them as radiopharmaceuticals,[1-4] we found that intramuscularly transplanted Ehrlich tumor was clearly visualized in scintigrams of mice a few hours after the administration of ^{99m}Tc complex of ethylenediamine-*N*,*N*-diacetic acid (Tc-99m EDDA). This finding prompted us to study the *in vivo* behavior and scintigrams of Tc-99m EDDA in mice and other animals bearing various experimental tumors. The present paper describes results which indicate that Tc-99m EDDA is a promising new radiopharmaceutical for tumor scintigraphy.

Experimental

^{99m}Tc EDDA——Ethylenediamine-*N*,*N*-diacetic acid (EDDA) was prepared according to the reported method,[5,6] and was complexed with ^{99m}Tc by the $SnCl_2$ method as described previously.[2,4]

Experimental Tumors——Animals bearing tumors were obtained by the following procedures.

Ehrlich Tumor: A series of ICR mice (body weight, *ca.* 30 g) were implanted with Ehrlich tumor cells (4×10^7 cells, freshly prepared from the ascites 7 d after inoculation) in the right foreleg and left for a period of 2—3 weeks to allow tumor growth.

Sarcoma 180: Sarcoma 180 cells were transplanted intramuscularly in the right foreleg of ICR mice (body weight, *ca.* 30 g) by a procedure similar to that used for Ehrlich tumor.

Lymphoma: Homotransplantable hamster lymphoma cells were inoculated into golden hamsters (body weight, *ca.* 150 g) in the right foreleg or right breast in a dose of 1×10^6 cells. The hamsters were left for a period of 2 weeks.

3-Methylcholanthrene (MC) Induced Fibrosarcoma: MC-induced fibrosarcoma (FC9-100) in a Fischer 344 rat was passed in syngeneic female rats by serial transplantation of a mechanically prepared single-cell tumor suspension. Intramuscular inoculation of 1×10^6 tumor cells in 0.2 ml HBSS (Hanks' balanced salt solution) into the right hind limb produced a tumor which metastasized spontaneously to the lung in 4 weeks. The tumor-bearing rats (body weight, *ca.* 300 g) were used 6 weeks after the inoculation.

C57 BL/6J mice were given injections of 1 mg of MC into the right hind leg muscles and developed fibrosarcoma. The tumor became palpable about 5 weeks after the injection of MC. The tumor-bearing mice (body weight, *ca.* 30 g) were used 8 weeks after the injection.

Spontaneous Mammary Carcinoma: Mice (C3H/HeN/JCL; body weight, *ca.* 30 g) bearing spontaneous mammary carcinoma were used.

Abscess: Mice (ICR) and rats were injected subcutaneously with 0.15 ml of a turpentine oil–liquid paraffin (1 : 1) mixture into the left foreleg and left for 1 d.

Inflammation: ICR mice were injected with 2% aqueous solution of carrageenan (0.15 ml) in the right foreleg and left for a period of 2 d.

Experimental Procedures——The animals were injected with 0.1 ml of Tc-99 m EDDA solution containing 500 μCi of ^{99m}Tc and *ca.* 1 mg of EDDA. The injection was done intravenously through the tail vein for mice and rats and intraperitoneally for hamsters. Sequential scintigrams were made at predetermined intervals with a scintillation camera (Toshiba GCA 202) having a pin hole collimator.

The mice bearing Ehrlich ascites tumor and Sarcoma 180 and the golden hamsters bearing lymphoma were sacrificed at selected times. All organs, some muscles and tumor tissues were removed and weighed, and the radioactivity was measured with an auto gamma scintillation spectrophotometer (Packard 5360). The percentage of the injected dose per gram of organs and tissues and the tumor/blood and tumor/muscle ratios of radioactivity were calculated.

Toxicity Study——The acute toxicity of EDDA was determined using three male mice weighing 30 ± 2 g each. EDDA was dissolved in saline and the pH of the solution was adjusted to 7.2 with aqueous NaOH. A dose of 1200 mg/kg EDDA was administered *i.p.* to each mouse. The mice were followed up for 30 d with normal animal care.

Results

Figure 1 shows scintigrams of mice bearing Ehrlich tumor after the administration of Tc-99 m EDDA. The ^{99m}Tc radioactivity was cleared from the blood and the image of the tumor became visible in 1 h. The image was very clearly visualized on the scintigram 2—5 h after the

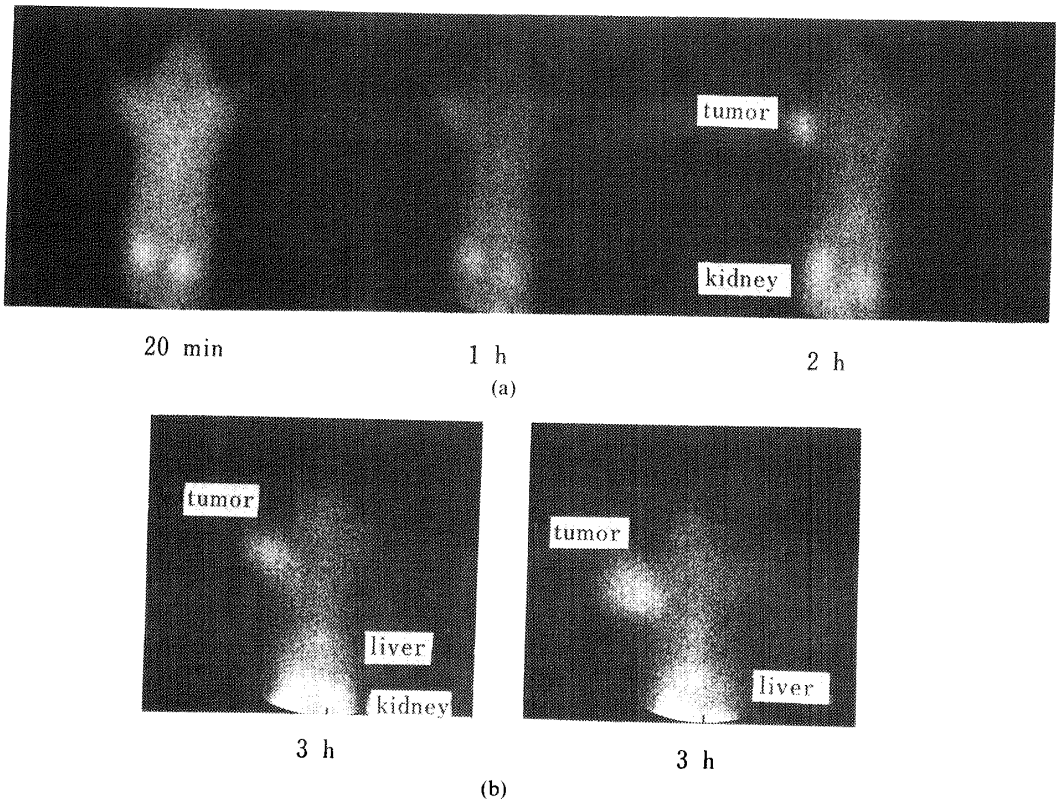


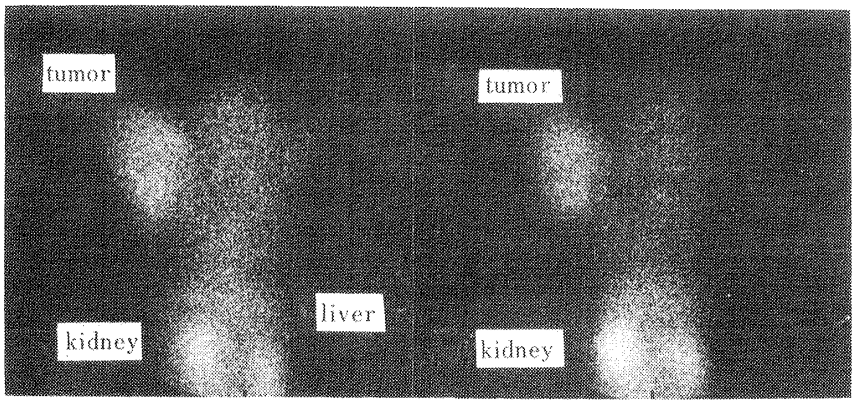
Fig. 1. Scintigrams of Mice Bearing Ehrlich Tumor with Tc-99 m EDDA (Anterior Projection)

(a) Sequential scintigrams at 20 min, 1, and 2 h after administration. (b) Scintigrams at 3 h.

TABLE I. Distribution of Radioactivity in Mice Following Injection of Tc-99 m EDDA

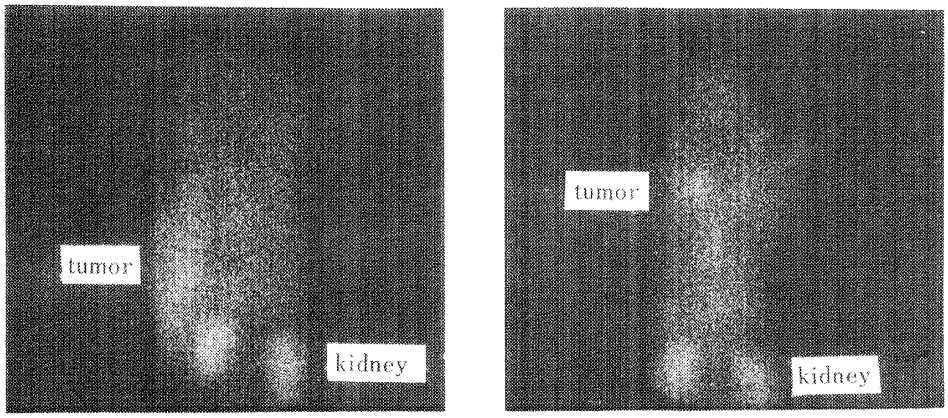
Organ	1 h	3 h
Blood	2.59 (2.21—2.91)[a)]	1.32 (0.97—1.56)
Muscle	1.02 (0.71—1.22)	0.55 (0.38—0.66)
Ethrlich tumor	4.89 (4.08—5.43)	4.38 (3.38—5.42)
Liver	1.92 (1.69—2.21)	2.22 (1.82—2.77)
Spleen	0.79 (0.71—0.87)	0.82 (0.74—0.93)
Stomach	1.81 (1.36—2.28)	1.72 (1.31—2.02)
Intestine	1.38 (1.05—1.78)	1.23 (0.89—1.45)
Kidney	18.69 (16.57—20.51)	16.65 (13.21—19.64)

a) Normalized mean % dose/g (and range) in three animals.



1.5 h 3 h

Fig. 2. Scintigrams of a Mouse Bearing Sarcoma 180 at 1.5 and 3 h



2 h 5.5 h

Fig. 3. Scintigrams of a Golden Hamster Bearing Lymphoma at 2 and 5.5 h

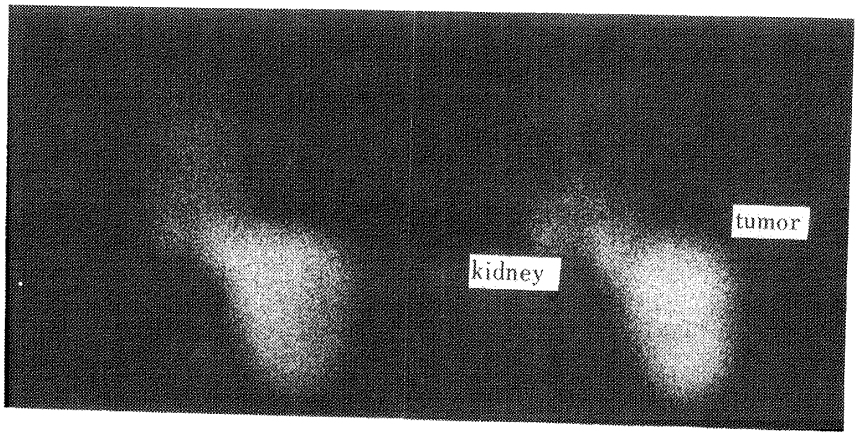
administration. The thyroid gland, stomach, and intestine were not visualized. ^{99m}Tc excreted in urine was proved to be in the form of the EDDA complex by means of thin layer chromatography (TLC). These results suggest that inorganic technetium was not liberated *in vivo*.

The results of the *in vivo* distribution study of Tc-99 m EDDA in the mice bearing Ehrlich

TABLE II. Distribution of Radioactivity in Mice and Golden Hamsters Following Injection of Tc-99m EDDA

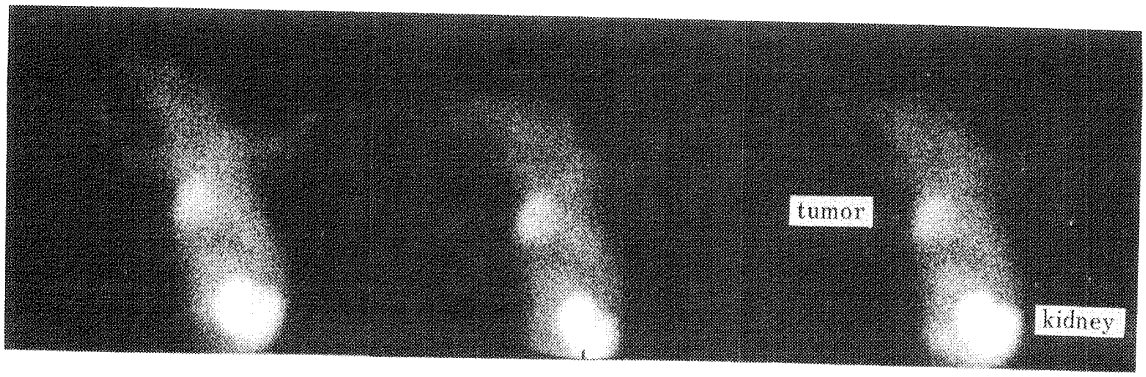
Organ	Mice (Sarcoma 180)	Golden hamsters (Lymphoma)
Blood	1.62 (1.43—1.84)[a)]	1.67 (1.29—1.97)
Muscle	0.48 (0.33—0.59)	0.51 (0.38—0.70)
Tumor	1.78 (1.48—2.01)	3.24 (2.86—3.56)
Liver	1.90 (1.58—2.23)	1.78 (1.39—2.21)
Spleen	0.87 (0.59—1.08)	1.05 (0.69—1.25)
Stomach	1.15 (0.75—1.48)	1.21 (0.97—1.51)
Intestine	1.23 (0.99—1.54)	1.46 (1.06—1.90)
Kidney	15.31 (12.89—19.66)	15.42 (13.21—18.89)

a) Normalized mean % dose/g (and range) in three animals. 3 h after the administration.



45 min 3.5 h

Fig. 4. Scintigrams of a Mouse Bearing Fibrosarcoma Induced by 3-Methylcholanthrene (MC) at 45 min and 3.5 h

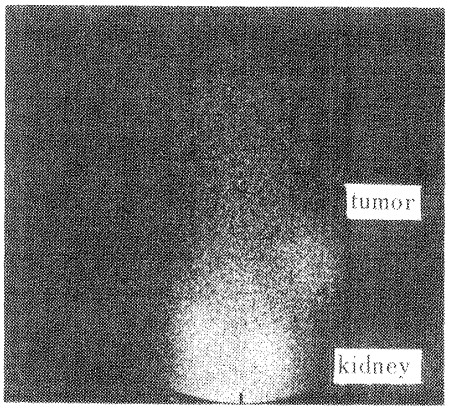


2 h 3 h 4 h

Fig. 5. Sequential Scintigrams of a Fischer Rat Bearing MC-Induced Fibrosarcoma Metastasized to the Lung at 2, 3, and 4 h

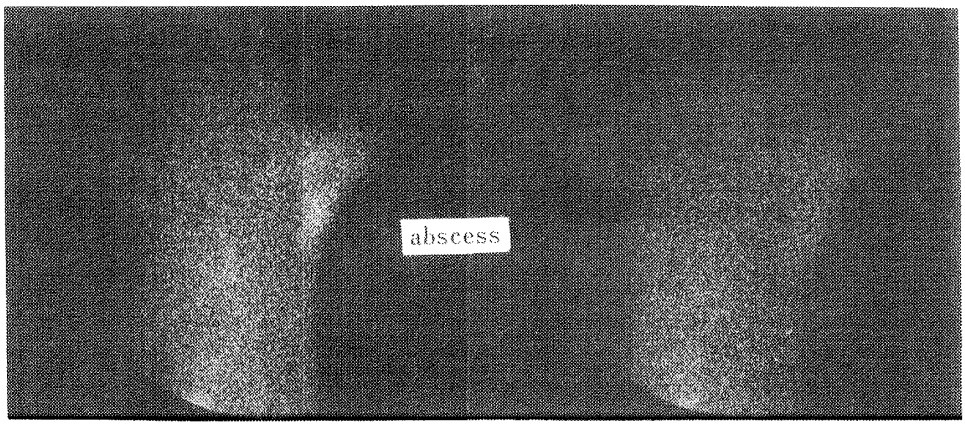
tumor 1 and 3 h after the administration are tabulated in Table I. Very high Tc uptake by the tumor and the kidneys is apparent. The ratios of the radioactivity in tumor/blood were 1.89 and 3.32 and those in tumor/muscle were 4.79 and 7.96, at 1 and 3 h after the administration, respectively. These values are quite satisfactory for tumor imaging.

When Tc-99m EDDA was administered intraperitoneally, clearance of the radioactivity from the blood was slower. It took more than 1 h before the image of the tumor became clear.



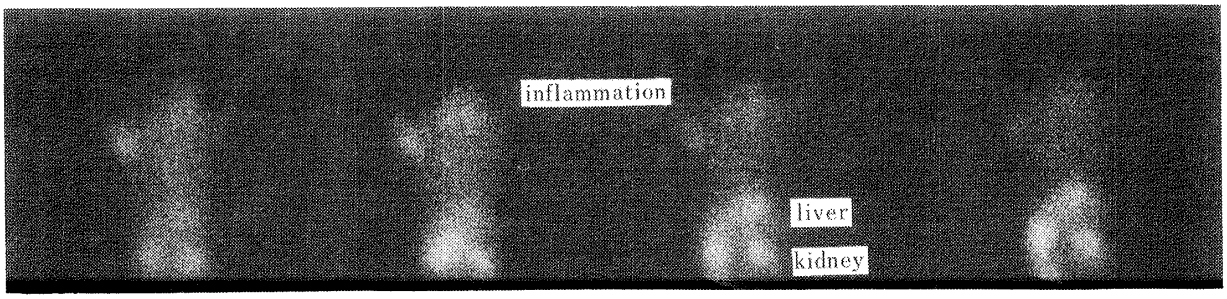
3 h

Fig. 6. A Scintigram of a Mouse Bearing Spontaneous Mammary Carcinoma at 3 h



2 h 4 h

Fig. 7. Scintigrams of a Mouse with an Abscess at 2 and 4 h



1.5 h 2 h 3 h 4 h

Fig. 8. Sequential Scintigrams of a Mouse with an Inflammation at 1.5, 2, 3, and 4 h

In animals that did not bear a tumor, the radioactivity was not accumulated in any specific organ and was excreted through the kidneys. The distribution and scintigrams in golden hamsters and dogs were reported previously.[1] The image of the pancreas was not visible in the scintigrams. Low uptake of the activity by the pancreas indicates that Tc-99 m EDDA did not behave as an α-amino acid.[1,7-9] High radioactivity was found in the kidneys, which suggests that scintigraphic visualization of tumors in this neighborhood would be prevented.

Scintigrams of a mouse bearing Sarcoma-180 and of a golden hamster bearing lymphoma

are shown in Figs. 2 and 3, respectively. The images of these tumors became visible 1.5 h after the administration of Tc-99 m EDDA and were clearly observed for several hours. The results of the distribution study in these animals at 3 h after the administration are summarized in Table II.

The tumor images were also very clear in scintigrams of a mouse and a Fischer rat bearing MC induced tumors as shown in Figs. 4 and 5. The lung metastases was scintigraphed clearly in the Fischer rat. The image of spontaneous mammary carcinoma was also visualized in a scintigram of a mouse as shown in Fig. 6, though the uptake of ^{99m}Tc by the tumor was smaller than that by the other experimental tumors.

The abscess caused by a turpentine oil–liquid paraffin mixture was visualized 2 h after the administration of Tc-99 m EDDA. The image was somewhat obscured 4 h after the administration (Fig. 7). The image of the carrageenan-induced inflammation in mice was visible at 2 h and was obscured 4 h after the administration (Fig. 8).

In the toxicity study, no animal died during the test period even at doses 3600 times those applicable to human patients.

Discussion

The present results indicate that the new radiotracer ^{99m}Tc EDDA gives satisfactory scintigrams of various experimental tumors in animals. Though the radioactivity was accumulated in areas of abscess and inflammation initially, their scintigraphic images were faded within a few hours. The images of the tumors persisted for several hours.

Studies on the mechanism of the accumulation in the tumors are in progress in our laboratory. No radioactivity was concentrated in the tumors by the administration of complexes of EDDA with ^{67}Ga, ^{51}Cr and ^{59}Fe, or ^{3}H labeled EDDA. The details of the studies will be reported elsewhere.

For scintiscanning, ^{99m}Tc appears to be an ideal radionuclide, giving good quality scans with only short intervals between the administration and the scanning. For these reasons, ^{99m}Tc is preferable to ^{67}Ga and ^{201}Tl, which are in current clinical use in tumor scintigraphy. Though the present work has proved that ^{99m}Tc EDDA is a promising radiotracer, much further work is required to define the effectiveness of this reagent in relation to that of other tumor specific agents in routine clinical use. Such work is in progress.

References

1) Part IV: Y. Karube, T. Maeda, M. Ohya, S. Sugata, A. Kono, and Y. Matsushima, *J. Radiat. Res.*, **23**, 234 (1982).
2) Y. Karube, A. Kono, T. Maeda, M. Ohya, and Y. Matsushima, *J. Nucl. Med.*, **22**, 619 (1981).
3) Y. Karube, T. Maeda, M. Ohya, A. Kono, and Y. Matsushima, *Chem. Pharm. Bull.*, **29**, 2385 (1981).
4) Y. Karube, T. Maeda, T. Imoto, M. Ohya, S. Sugata, A. Kono, H. Okano, and Y. Matsushima, *Chem. Pharm. Bull.*, **30**, 2529 (1982).
5) R. M. Genik-Sas-Berezowsky and I. H. Spinner, *Can. J. Chem.*, **48**, 163 (1970).
6) G. McLendon, R. M. Motekaitis, and A. E. Martell, *Inorg. Chem.*, **14**, 1993 (1975).
7) F. Friedberg, H. Tarver, and G. M. Greenberg, *J. Biol. Chem.*, **173**, 355 (1948).
8) M. Blau and R. F. Manske, *J. Nucl. Med.*, **2**, 102 (1961).
9) K. F. Huebner, G. A. Andrews, E. Buonocore, R. L. Hayes, L. C. Washburn, I. R. Collmann, and W. D. Gibbs, *J. Nucl. Med.*, **20**, 507 (1979).