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LABELING OF MAMMALIAN NUCLEATED CELLS WITH ^{99m}Tc

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A rapid and simple method for labeling mammalian nucleated cells with ^{99m}Tc is described. A wide variety of tissue culture cells of mouse and human origin as well as lymphocytes isolated from peripheral blood have been labeled without appreciable loss of viability. Optimum labeling was obtained by incubating viable cells suspended in a balanced salt solution with 10 mCi ^{99m}Tc and 100 μg Na_2CrO_4 for 15 min at 37°C followed by reduction with 0.2% $SnCl_2$ solution. Serum protein interfered with the labeling process. 10,000 viable cells gave between 1.2 and 6 × 10^5 cpm immediately after labeling. Mammalian nucleated cells retain approximately 90% of the bound radioisotope when injured or killed, and that which is released does not appear to be in a reusable form. Labeling with ^{99m}Tc had no consistent effect on the cells' ability to synthesize DNA or proteins.

Technetium-99m has been used to label mammalian erythrocytes (*1–5*). The simplicity of the procedure suggested that it also might be applicable to nucleated cells, and we now describe a method by which a variety of somatic cells can be rapidly and easily labeled with this radionuclide.

MATERIALS AND METHODS

The procedure initially was applied to Sarcoma I (Sa I), a transplantable murine fibrosarcoma of strain A/J origin (*6*). The tumor was established in tissue culture and maintained in Eagle's minimum essential medium supplemented with 10% newborn calf serum. Tumor cells growing in monolayers were harvested by incubation for 30 min at 37°C with a 0.25% solution of trypsin in calcium and magnesium-free phosphate buffered saline (pH 7.4). The cells were washed twice in Hanks' balanced salt solution (HBSS) and their final concentration adjusted to 1–1.5 × 10^6/ml. Ten to 15 mCi of sterile $Na^{99m}TcO_4$ solution were added to 10–15 million viable cells that were suspended in 10 ml of HBSS containing 100 μg of sodium chromate (Na_2CrO_4). The $Na^{99m}TcO_4$ was obtained by eluting a New England Nuclear molybdenum-technetium generator with 0.9% saline. After 15–20 min incubation at 37°C with occasional mixing, the pertechnetate was reduced by adding 0.2–0.3 ml of a sterile solution of 0.2% stannous chloride ($SnCl_2 \cdot 2H_2O$) in drops with constant stirring (*4,7*). The stannous chloride was dissolved in freshly prepared acid-citrate dextrose (30 gm $Na_3C_6H_5O_7$, 0.15 gm NaH_2PO_4, and 2.0 gm dextrose per liter of distilled H_2O, pH adjusted to 7.4) that was sterilized by passage through a 0.22 micron Millipore filter. Following an additional 15-min incubation at 37°C, the cells were sedimented by centrifugation at 400 G for 10 min and the unbound isotope removed by washing them three times with 35-ml aliquots of HBSS. In order to obtain living labeled cells it was important to use a cell preparation of high initial viability and to maintain the pH of the reaction mixture at 6.5–7.0. Since dead cells are labeled equally as well as living ones, the final cell suspension contained a mixture of labeled viable and nonviable cells, with viability ranging from 80–95%.

RESULTS

Table 1 shows the results of labeling 2.6 × 10^6 viable Sa I cells with amounts of ^{99m}Tc varying from 0.3 to 10.0 mCi. The radioactivity, determined with a Nuclear-Chicago Model 1185 gamma scintillation counter, is expressed as the mean counts

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TABLE 1. LABELING EFFICIENCY OF SARCOMA I AS RELATED TO MILLICURIES OF ^{99m}Tc USED*

^{99m}Tc (mCi)	Mean cpm ± s.e.
0.3125	1,455.5 ± 53.4
0.6250	3,209.3 ± 259.5
1.250	12,881.0 ± 445.7
2.500	24,453.7 ± 705.9
5.000	39,207.7 ± 602.4
10.000	73,865.5 ± 1,625.3

* Radioactivity was determined in a Nuclear-Chicago Model 1185 gamma scintillation counter. Triplicate samples of 8,000 viable cells each were counted 12 hr after labeling, and background has been subtracted.

TABLE 2. LABELING EFFICIENCY OF SARCOMA I AS RELATED TO THE PRESENCE OF SODIUM CHROMATE AND STANNOUS CHLORIDE IN THE REACTION MIXTURE

Sa I	Volume of Hanks' BSS	Na_2CrO_4	^{99m}Tc (mCi)	$SnCl_2 \cdot 2H_2O$	CPM of cell pellet*
2×10^6	10 ml	100 μg	2.0	600 μg	243,690
2×10^6	10 ml	0	2.0	600 μg	171,720
2×10^6	10 ml	0	2.0	0	5,710
2×10^6	10 ml (10% serum)	0	2.0	600 μg	27,560

* Radioactivity of cell pellets washed three times was determined 24 hr after labeling with a Picker Spectroscaler IIIA in conjunction with a Picker Nuclear Omniwell.

per minute ± standard error for triplicate samples of 8,000 viable cells each. The amount of radioisotope incorporated into a given number of nucleated cells was linearly related to the starting amount of ^{99m}Tc (*8*), and maximum labeling was achieved with 10 mCi. Varying the reaction conditions could substantially alter the labeling efficiency (Table 2). Aliquots of 2×10^6 Sa I cells were labeled with 2.0 mCi ^{99m}Tc, using four different protocols to assess the effect that sodium chromate, stannous chloride, and serum proteins had on labeling efficiency. The addition of Na_2CrO_4 increased the labeling efficiency by approximately 30%. This has been attributed to a carrier effect whereby $CrO_4^=$ facilitates the entry of $^{99m}TcO_4^-$ into the cell (*3*). Stannous chloride was essential to obtain optimum labeling. Serum proteins markedly inhibited labeling, possibly by providing a substrate to which the pertechnetate was attached by reduction.

DISCUSSION

Using the above procedure it has been possible to label tumor cells obtained from murine fibrosarcoma (Sa I), neuroblastoma (C 1300), and melanoma (B 16) as well as human carcinomas of the lung, breast, and colon, osteogenic sarcoma, melanoma, and neuroblastoma (Table 3). Depending upon the cell type used, 10,000 viable labeled cells gave between 1.2 and 6×10^5 cpm immediately after labeling. In addition to cells grown in tissue culture, it has been possible to label human peripheral blood lymphocytes isolated on a Ficoll-Hypaque gradient (*9*) and Sarcoma I that has been maintained in ascitic form in A/J mice. The ease with which nucleated mammalian cells can be successfully labeled with ^{99m}Tc suggests many in vivo and in vitro applications. Intravenous injection of 10^6 viable labeled Sa I cells in mice has provided information on the localization of circulating tumor cells. The radioactivity of 10^6 viable labeled tumor cells injected intravenously into mice was sufficient to be detected by a Nuclear-Chicago Pho/Gamma HP scintillation camera. Although scanning resolution was poor due to the small size of the animal, high counts for liver, spleen, and lung were obtained by removing these organs and counting them separately in a well counter. In contrast to ^{99m}Tc-labeled erythrocytes that release a very large percentage of the radioisotope when lysed (*3*), nucleated mammalian cells retain approximately 90% following cyclic freezing and thawing. This fact, coupled with our observation that the isotope was not released in a reusable form by dead or injured cells, has led us to use ^{99m}Tc-labeled target cells for the in vitro assessment of cell-mediated immunity (*8,10–13*) and lymphotoxin activity (*14*). In comparison with the ^{3}H-thymidine (*15*) and ^{125}I-5-iodo-2′-deoxyuridine (*16*) radioisotopic assays and one based on

TABLE 3. MAMMALIAN CELL TYPES LABELED WITH ^{99m}Tc

Code	Cell or histopathologic type	Species of origin
L 929	Normal fibroblast	Mouse (C3H)
B 16	Melanoma	Mouse (C57B1/6)
C 1300	Neuroblastoma	Mouse (A/J)
Sa I	Fibrosarcoma	Mouse (A/J)
HT_{71}^{-1}	Bronchogenic carcinoma	Human
HT_{71}^{-43}	Normal lymphocytes	Human
HT_{71}^{-43}	Normal lymphocytes	Human
HT_{71}^{-44}	Carcinoma of the breast	Human
HT_{71}^{-45}	Adenocarcinoma of the colon	Human
HT_{71}^{-66}	Osteogenic sarcoma	Human
HT_{71}^{-67}	Malignant melanoma	Human
HT_{72}^{-68}	Malignant melanoma	Human
HT_{72}^{-69}	Malignant melanoma	Human
HT_{72}^{-76}	Malignant melanoma	Human
HT_{72}^{-77}	Malignant melanoma	Human
HT_{72}^{-78}	Neuroblastoma	Human
HeLa	Carcinoma of cervix	Human
	Peripheral blood lymphocytes	Human

visual enumeration of surviving cells (*17,18*), the ^{99m}Tc microcytotoxicity assay was simple to perform yet possessed equal or greater sensitivity and reproducibility. The short labeling time and strong binding affinity of the radionuclide more than compensated for its short half-life. The labeling of cells did not appear to alter either their capacity to synthesize DNA as determined by the incorporation of ^{3}H-thymidine or to synthesize proteins as determined by the incorporation of ^{3}H-labeled amino acids (*19*). The ability to label almost any type of mammalian nucleated cell with ^{99m}Tc is an important methodological advance with wide applicability to in vivo and in vitro systems.

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