Generator-Produced Yttrium-90 for Radioimmunotherapy

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Yttrium-90 is often considered to possess many favorable properties for radioimmunotherapy applications. Among these is its availability as a radionuclide generator product by decay of its parent, ^{90}Sr. Nevertheless, most present and planned clinical trials with ^{90}Y-labeled antibodies employ radioactivity obtained not from an in-house generator, but from commercial sources. To prepare for clinical trials at this institution with ^{90}Y labeled to diethylenetriaminepentaacetic acid- (DTPA) coupled antibodies, we have adapted previously published procedures and have developed others to prepare antibodies labeled with generator produced ^{90}Y for human use. Up to 25 mCi of ^{90}Sr have been loaded without evidence of radiolytic degradation to the Dowex 50 cation exchange resin which serves as the solid support for the generator. Using 0.003*M* ethylenetriaminetetraacetic acid (EDTA) as eluant, elution efficiency averages 98% and ^{90}Sr breakthrough averages 0.002%. The EDTA is destroyed remotely and the activity is dissolved in 0.5*M* acetate, pH 6. In this form, ^{90}Y may be used to label DTPA-coupled proteins at specific activities of 1–3 mCi/mg (an order of magnitude improvement in specific activity results from the purification of ^{90}Y by cation exchange prior to labeling). When properly labeled, size exclusion HPLC shows 90% or greater radiochemical purity and recovery without postlabeling purification. We conclude that these techniques provide a ^{90}Y-labeled protein preparation which is safe for administration to patients.

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Yttrium-90 (^{90}Y) is often believed to be among the most useful of the radionuclides that have been considered for radioimmunotherapeutic applications (*1*). This radionuclide has a half-life (64 hr) consistent with the rate of antibody accumulation in tumor, no accompanying gamma-ray radiation in its decay, beta rays of intermediate energy, and a stable daughter. Furthermore, it has been shown that antibodies may be labeled with ^{90}Y through attached diethylenetriaminepentaacetic acid (DTPA) groups (*2–4*). It is significant that ^{90}Y may be available conveniently and inexpensively as a radionuclide generator product by decay of its 28-yr parent, strontium-90 (^{90}Sr). Although several ^{90}Y generator systems of varying design have been described, most clinical studies of ^{90}Y-labeled antibodies have been performed with activity obtained not from in-house generators, but from commercial sources (*5*). Since the use of generator-produced ^{90}Y would substantially reduce the cost of this radionuclide and, at the same time provide activity on demand, extensive use of this radionuclide may require the development of methods for the labeling of antibodies with ^{90}Y obtained from generators.

Although solvent extraction methods for the separation of ^{90}Y from ^{90}Sr have been reported, it is the ion exchange methods that have received the most attention regarding generator construction (*6*). Numerous methods have been reported where the cation exchange resin Dowex 50 has been used to retain ^{90}Sr while the daughter ^{90}Y activity is eluted with lactate (*7*), methanol and acetate (*8*), oxalate (*9*), citrate (*10*), and ethylenetriaminetetraacetic acid (EDTA) (*6,11,12*). A disadvantage common to all of the above is that the activity is not eluted in ionic form in a condition suitable for direct labeling of DTPA-conjugated proteins. The concentration of complexing and chelating agents present in these eluants is such that under many circumstances a competition will exist with the DTPA groups for the activity with a resulting decrease in labeling efficiency. Accordingly, it will usually be necessary to remove these agents

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prior to protein labeling to avoid the need for postlabeling purification.

A particularly important consideration in a generator system intended for clinical use is the degree of ^{90}Sr breakthrough. Reports of Doering et al. (*13*) and Skraba et al. (*6*) have accurately determined this parameter. The latter generator system was eluted with 0.003*M* EDTA, pH 4.6 and displayed a breakthrough of <0.1% while the former was eluted with 0.03*M* citrate, pH 5.5 and resulted in an exceedingly low breakthrough of 10^{-6}%. However, this low level of ^{90}Sr breakthrough is due, at least in part, to the large 4-cm-wide and 18-cm-long resin column used in this generator. The 100-ml elution volume required for good ^{90}Y yields in impractically large for subsequent use for protein labeling. By contrast, the EDTA generator of Skraba et al. was of more modest dimensions, measuring only 0.6 cm in width and 1.0 cm long, and required only 5-ml elution volumes. When the size of the citrate generator was reduced to these dimensions, approximately twice the elution volume was required for adequate ^{90}Y yield and we found breakthrough to be in excess of that provided by the EDTA generator (unpublished data).

This investigation, like our previous study (*2*), was conducted with the EDTA generator of Skraba et al. This report describes experiences in the construction, elution, and use of this generator for the preparation of ^{90}Y-labeled antibodies. Since these antibodies are intended for human administration, labeling was by "kit" methods in which postlabeling purification was not performed.

MATERIALS AND METHODS

All reagents were analytical grade and the water used in this investigation was purified on a Milli-Q system.*

Generator Construction

Figure 1 shows the generator system with lead removed. The generator consists of a glass column with a 0.8 cm diameter and 16 cm long with a frit at the bottom and fitted at both ends with a plug containing a small diameter tubing. The column is mounted vertically and is surrounded with 5 cm of lead.

The Dowex 50 W × 8 (50–100 mesh)† cation exchange resin was first washed with 1*M* NaOH to convert the resin to the sodium form and was then washed free of excess base with distilled water. Twenty-five millicuries of ^{90}Sr‡ in 6.2 ml of 1*M* HNO_3 was added to a 50-ml beaker along with 0.75 ml of 0.03*M* EDTA.§ Approximately 0.66 ml of 10*M* NaOH was required to raise the pH to 4.5. Five grams of the cation exchange resin was then added and the suspension stirred slowly overnight. The pH of the suspension was unchanged throughout. The glass column was then loaded, first with 1 g of the resin without radioactivity followed by the 5 g of resin containing the ^{90}Sr. The generator was then washed with several column volumes of 0.003*M* EDTA, pH 4.6, to remove the traces of unbound ^{90}Sr.

The generator was placed in a fume hood. The inlet tube was connected to a peristaltic pump while the outlet tube was attached to a small diameter glass tube within a Pasteur pipette suspended above an acid-washed conical quartz vial 1.6 cm in diameter and 9.0 cm long. The quartz vial was wrapped in heating tape and placed within a lead shield.

During each elution, 5 ml of 0.003*M* EDTA, pH 4.6, was forced through the generator and into the quartz vial at a flow rate of 0.7 ml/min by means of the peristaltic pump. The solution was then evaporated in ~8 min by applying current to the heating tape by means of a Variac.¶ To speed evaporation, a second Pasteur pipette was suspended above the vial and used to blow a gentle stream of nitrogen gas—filtered free of metallic particles—on the evaporating liquid. Following evaporation to dryness, 8 ml of 1:1 conc. H_2SO_4:HNO_3† was added to the vial and the contents evaporated to dryness, in ~20 min to destroy the EDTA. After the vial cooled, the ^{90}Y activity dissolved in the desired quantity (usually 0.1 ml) of sterile, pyrogen-free 0.5*M* acetate, pH 6.0. No loss of ^{90}Y activity due to volatilization was observed during the evaporations.

Strontium-90 Breakthrough

A rapid estimate of ^{90}Sr breakthrough may be obtained by the paper chromatographic technique described previously (*2*). On Whatman No. 1 paper and with saline eluant, ^{90}Sr migrates with the solvent front while ^{90}Y in the acetate form remains near the origin. In the absence of ^{90}Y as the EDTA chelate, the ^{90}Sr breakthrough may be estimated from the radioactivity at the solvent front versus that of the entire chromatogram. Because this assay is completed in <1 hr, it may be performed routinely on each preparation of ^{90}Y acetate prior to patient administration as a check against catastrophic generator failure.

A more involved and accurate determination of breakthrough is that of Doering et al. (*13*) where an anion-exchange resin retains most of the ^{90}Y while allowing the ^{90}Sr to be eluted for counting. To an aliquot of ^{90}Y acetate solution containing ~1 mCi of activity, 2 mg of strontium and 10 mg of yttrium carrier are added. The solution is then added to the top of a 1-cm-diameter by 16-cm-long anion-exchange column containing AG 1 × 4 (50–100 mesh)** in the hydroxide form. The column is then eluted with 10 ml of water into a 10-ml volumetric flask. The flask is filled up to the mark, the contents mixed by agitation, and exactly 1 ml removed for evaporation to dryness on a planchette. The planchette is then counted at fixed geometry on the end-window GM†† counter with and without a 220 mg/cm^2 aluminum absorber. The mica window on the GM counter is of a thickness which does not appreciably absorb the beta rays of either ^{90}Sr or ^{90}Y (see Results) and the aluminum attenuates all the soft beta rays from ^{90}Sr and ~50% of the harder beta rays from ^{90}Y. A standard containing 0.01 μCi of ^{90}Sr-^{90}Y, also mounted on a planchette,‡‡ is counted in the same manner and at the same geometry.

Yttrium-90 Purification

An important goal of this investigation was to avoid the need for postlabeling purification since the labeled antibodies are intended for human use. Using antibodies conjugated with an average of 1–2 DTPA groups per molecule, we routinely obtain specific activities of 1–3 mCi/mg of labeled protein

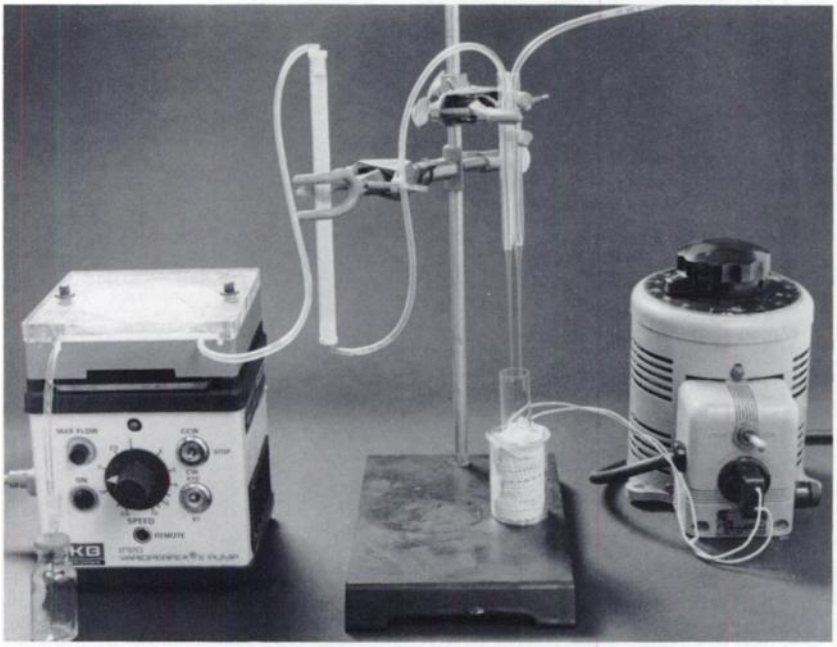

FIGURE 1
Photograph of the generator system with lead removed.

with 90% or greater radiochemical purity. Under normal circumstances, attempts to label at much higher specific activities results in a high percentages of free ^{90}Y and therefore a lower radiochemical purity.

Higher specific activities may be attained, however, following purification of the ^{90}Y activity from excessive trace metal contamination. We have employed the procedure of Strelow et al. (*14*) for this purification. The ^{90}Y, either as the acetate or the EDTA chelate, is made 0.5*M* in H_2SO_4 and added to a 1-cm-diameter by 20-cm-long cation exchange column of AG 50W × 4 (100–200 mesh)** in the hydrogen form. The column is washed with 10 ml of 0.5*M* H_2SO_4 and then with 50 ml of 1.8*M* HCl. The activity is then eluted into an acid-washed beaker with 25 ml of 4.0*M* HCl. Thereafter, the HCl solution is evaporated to dryness in the quartz vial and the activity converted to the acetate as before.

Antibody Labeling

DTPA-coupled antibodies may be radiolabeled with ^{90}Y in a similar manner to indium-111 (^{111}In) (*15*) by adding the activity as the acetate complex. To avoid diluting the protein (and thereby lowering the DTPA concentration), the activity is added in as small a volume as possible. After 10–30 min, the radiochemical purity is determined by size exclusion high performance liquid chromatography (HPLC). In addition to the determination of radiochemical purity, the recovery of radioactivity from the HPLC is also determined routinely.

Antibody Radiolysis

Since in practice it is often necessary to store radiolabeled antibodies intended for patient administration for periods of up to several hours, it is important to determine whether radiolysis of the antibody occurs during this period. It has been shown previously by affinity chromatography that activity concentrations of ^{90}Y of 2 mCi/ml had no affect on the ability of the 19-9 antibody to bind to its antigen (*2*). This investigation has been repeated at the higher activity concentrations likely to be encountered in practice.

The affinity column was essentially identical to that previously reported (*2*) and consisted of 19-9 antibody immobilized on a Sepharose support to which the antigen is added in a cell supernatant. Being multifunctional, the bound antigen is capable of binding additional 19-9 antibody. A 0.5-cm-diameter by 3.0-cm-long column of the resin was prepared.

To each of the two test tubes, 20 μg of 19-9 $F(ab')_2$ antibody containing an average of 1.8 DTPA groups per molecule was added followed by 100 μCi of ^{111}In acetate to label the protein. Both test tubes received 300 μl of 0.8*M* acetate buffer and, in one test tube only, 7.2 mCi of ^{90}Y as the acetate. Thus, the initial radioactivity concentration of this sample was 23 mCi/ml with respect to ^{90}Y. Each preparation was analyzed at two time points, once immediately after mixing and again after storage at room temperature for 24 hr. Analysis consisted of adding to the affinity column 2 μl of each preparation, diluted to 0.4 ml with 0.05*M* citrate buffer, pH 4.0, and collecting 0.5 ml fractions during elution with the buffer. The ^{111}In activity was determined in the presence of ^{90}Y activity by counting each fraction on an intrinsic germanium gamma-ray detector§§ and summing the intensity of both gamma-ray peaks in the decay of ^{111}In. Each sample was analyzed four times at each time point along with a standard. In this manner, the percentage of ^{111}In bound to the column was determined.

Animal Studies

Biodistribution studies in CD-1¶¶ male mice were performed by tail vein administration of several monoclonal antibodies as well as nonspecific antibodies conjugated with DTPA and radiolabeled with ^{111}In or ^{90}Y. Mice were killed either at 1 hr or several days to investigate the relative biodistributions of the two labels on the same proteins. Limited biodistributions involving only blood and bone radioactivity level determinations were performed for preparations of DTPA-coupled IgG antibodies labeled only with ^{90}Y and displaying a range of radiochemical purities and recoveries determined by size exclusion HPLC.

RESULTS

The 25 mCi generator used in this research has shown no sign of radiolysis in the 6 mo since its construction as indicated by a constant elution efficiency (mean 98%, 2.5% s.d.; N = 11) and ^{90}Sr breakthrough determined at regular intervals since its construction. Following the procedure described above, the [^{90}Y]acetate may be used directly to radiolabel antibodies. At an average of 1–2 DTPA groups per protein molecule and at a protein concentration of 5 mg/ml, specific activities of 1–3 mCi/mg of protein are achieved with radiochemical purities of 90% or more. Following purification by cation exchange as described above and under identical labeling conditions, however, specific activities of 10 mCi/mg have been achieved with comparable radiochemical purities. In control studies where the DTPA anhydride was deliberately hydrolyzed to prevent protein conjugation, we have previously (*2*) and subsequently observed that under the present circumstances the ^{90}Y is bound exclusively to the DTPA groups on the antibodies considered herein. Maximum labeling of DTPA-coupled antibodies in this study required only ~30 min.

Using the method of Doering et al. (*13*), the ^{90}Sr breakthrough has been shown to be 0.002% of the ^{90}Y activity at the time of elution (0.003% s.d., N = 10). The end-window GM counter described in the previous section and used to obtain these measurements is fitted with a mica window between 1.0 and 1.2 mg/cm^2 in thickness. Since the range of the 0.55 MeV beta rays of ^{90}Sr in this material is ~180 mg/cm^2, the window will provide little attenuation of the beta rays of ^{90}Sr and, especially, the more energetic beta rays of ^{90}Y. This was confirmed by separating ^{90}Sr and ^{90}Y from an equilibrium mixture and counting each separately with the GM tube. The separation was achieved by paper chromatography as described above and the portion of the strip containing each activity mounted on planchettes. Repeat counts of each planchette over 35 days showed behavior similar to that observed earlier (*2*) in that the activity in the planchette containing ^{90}Y decreased while that in the planchette containing ^{90}Sr increased until equilibrium was attained about 10–12 days postseparation. The initial activities of both planchettes differed only by 16% and the maximum activity attained by the initially pure ^{90}Sr sample was equal within experimental error to the sum of both the initial activities. These observations show that this detector detects the soft beta rays of ^{90}Sr and the harder beta rays of ^{90}Y equally and may, therefore, be used in the determination of ^{90}Sr breakthrough.

Although the levels of ^{90}Sr in the ^{90}Y eluants is low, the contamination of ^{90}Y-labeled antibodies with ^{90}Sr may be reduced still further with postlabeling purification. Figure 2 shows a composite of three radiochromatograms obtained by the paper chromatographic analysis described above. One chromatogram is that of DTPA-coupled antibody labeled with ^{111}In and shows some migration of activity with the solvent front as expected for labeled proteins depending on protein loading (*16*). Another chromatogram is that of the same DTPA-coupled antibody preparation, but after labeling with an equilibrium mixture of ^{90}Sr-^{90}Y as the acetate. The two radiochromatograms are very similar with the exception that a radioactive peak now appears at the solvent front. That this peak is due to ionic ^{90}Sr may be surmised from the behavior of this element in this chromatographic system as described above and by the third radiochromatogram in the figure which was obtained by re-scanning the chromatogram 3 wk later after ^{90}Y had decayed. The absence of origin activity in this chromatogram shows that the DTPA groups on the antibody chelate only yttrium and not strontium (*4*). Accordingly, the level of ^{90}Sr in these ^{90}Y-labeled antibody preparations may be reduced by postlabeling purification methods such as gel filtration which separate strontium from the labeled antibody.

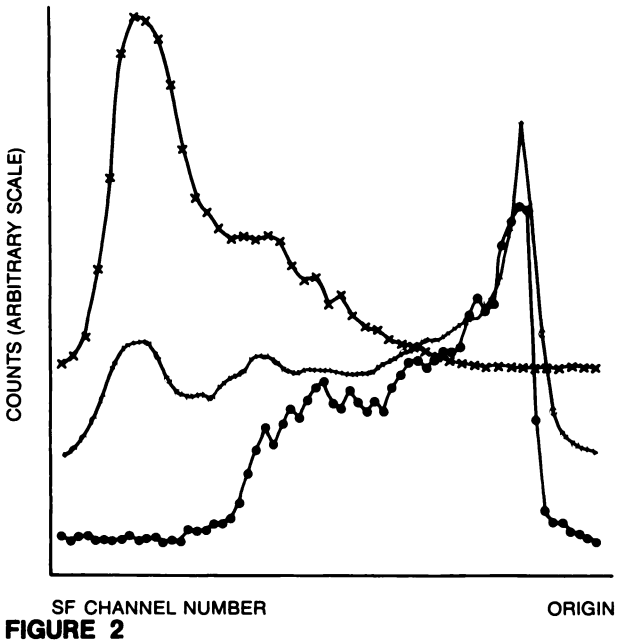

FIGURE 2
A composite of three radiochromatograms. The lowest chromatogram (●●●) obtained by analysis of a DTPA-coupled antibody labeled with ^{111}In. Middle chromatogram (○○○) obtained by analysis of the same DTPA coupled antibody immediately after labeling with an equilibrium mixture of ^{90}Sr-^{90}Y. Upper chromatogram (crosses) obtained by recounting the previous chromatogram after the decay of ^{90}Y. (SF = solvent front, OR = origin).

The possibility that the ^{90}Y eluants were contaminated with radionuclides other than ^{90}Sr was also investigated. A sample of eluant containing ~4 mCi of ^{90}Y activity was allowed to decay for 3 mo and an aliquot of the sample was then counted at fixed geometry on

the intrinsic germanium gamma-ray detector. The detector had been calibrated with gamma-ray standards such that the absolute detection efficiency at that geometry was determined for a range of gamma-ray energies. Following an extended count of the decayed sample, no gamma-ray peaks were observed above background. It is estimated from these results that an upper limit on radionuclide contamination other than ^{90}Sr is $\sim 10^{-7}$% of ^{90}Y activity at end of elution.

Regarding the effect of ^{90}Y activity on antibody immunoreactivity, ^{111}In binding to the affinity column was 39% (range 30–48%) initially and 44% (34–53%) at 24 hr in the absence of ^{90}Y and 37% (34–40%) and 33% (31–35%), respectively, in the presence of ^{90}Y. These results indicate that for one antibody at least, storage of ^{90}Y-labeled antibodies for reasonable periods should not present a problem.

Figure 3 shows that a relationship exists between blood levels of ^{90}Y in mice at 1 hr postinjection and radioactivity recovery determined by size exclusion HPLC. In those cases where trace metals were deliberately introduced into the DTPA-coupled protein preparation prior to radiolabeling, both low radioactivity recovery and low blood levels were observed even though for certain of these preparations radiochemical purity determined by HPLC remained high. Biodistribution studies indicate that the decreased blood levels may be accompanied by increased bone activity levels. It is possible, therefore, that low recovery indicates the presence of unbound ^{90}Y in a chemical form which clears blood rapidly and deposits in bone. That examination of the radioactivity trace obtained by size exclusion HPLC cannot at all times detect the presence of this unbound species suggests that it is prone to be retained by the HPLC column.

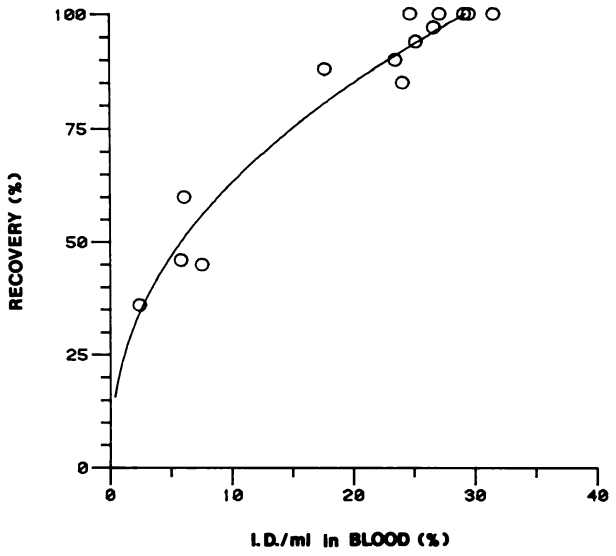

FIGURE 3
Relationship between the percent radioactivity recovered during size exclusion HPLC analysis of several ^{90}Y-labeled antibodies and the blood radioactivity levels at 1 hr post i.v. administration of these labeled antibodies to mice. (I.D. = injected dose)

DISCUSSION

Interest in the use of ^{90}Y in radiocolloid and ionic form for radiotherapy has been extensive in the past (*7–12*), and has resulted in several of the developments incorporated in these studies. Current interest in the use of monoclonal antibodies for the treatment of cancer has reawakened interest in ^{90}Y since, in addition to the advantages of this isotope that were recognized earlier, may be added the fact that ^{90}Y will form stable chelates with proteins modified by the bifunctional chelate approach (*2–4*).

Among the generator systems previously described, two appear to be particularly well characterized. The generator of Doering et al. (*10*) was commercially available for a time while that of Skraba et al. (*6*) is the most recent. Both employ the same cation exchange resin, the former using a citric acid eluant and the latter an eluant containing EDTA. Possibly because citrate is a weaker complexing species than EDTA, a larger volume of this eluant is required to achieve good ^{90}Y yields. When the same size generator was eluted with each eluant, 30 ml of 0.03*M* citrate eluant was required for 95% yield while 5 ml of 0.003*M* EDTA eluant routinely provides this or better yields. A large volume increases the time required to convert the radioactivity into a form suitable for protein labeling. More importantly, however, the ^{90}Sr breakthrough was found to be 0.1% in the citrate eluant and only 0.01% in the EDTA eluant. Thus, the EDTA generator appears to be more attractive on consideration of ^{90}Sr breakthrough alone. The annual limit on intake of ^{90}Sr by radiation workers has been set at 29 μCi of oral exposure of which a minimum of 20% may be absorbed (*17*). The ^{90}Sr intake to a patient receiving 20 mCi of ^{90}Y with a 0.002% breakthrough is only 0.4 μCi and therefore substantially below the above value.

A disadvantage of existing generators which rely upon a chelating or complexing agent to extract ^{90}Y from the cation exchange column is that the eluant may not be used directly for protein labeling under the conditions of this work. Although DTPA-coupled proteins may be radiolabeled directly, this will usually be at the expense of labeling efficiency. Both the low specific activity of ^{90}Y in the eluant, that will dilute the DTPA concentration of conjugated protein, and the presence of complexing or chelating agents, which will compete with DTPA for the activity, contribute to poor labeling. Since an important object of this investigation was to prepare labeled proteins suitable for human use, the use of postlabeling purification, necessary whenever labeling efficiency is low, had to be avoided. Consequently,

it became necessary to develop an apparatus capable of remotely and rapidly converting the 5 ml of 0.003*M* EDTA eluant to ~0.1 ml of 0.5*M* acetate without introducing trace metals at levels that would interfere with subsequent protein labeling. This was accomplished with the apparatus described above that, in ~1 hr, provides a ^{90}Y preparation capable of radiolabeling DTPA-conjugated antibodies at a specific activity of 1–3 mCi/mg with 90% or greater radiochemical purity. This specific activity has been acceptable in this research thus far (although a factor of 10 improvement in this value may be achieved by adding a preliminary cation exchange purification step for the ^{90}Y). In addition to adequate labeling, ^{90}Y prepared in the above fashion does not display the slow binding kinetics observed earlier (*2*) by us but binds to DTPA-coupled proteins in 10–30 min. Finally, when sterile and pyrogen-free acetate is used to dissolve the evaporated ^{90}Y, the resulting acetate solution has been found to be sterile and pyrogen free.

The biodistributions obtained for certain antibodies radiolabeled with ^{90}Y have confirmed our earlier observations (*2*) that identity with the biodistribution of ^{111}In on the same protein occurs only at early time points. Thereafter, some differences appear which may be related to redistribution of the labels following catabolism.

Of particular importance to the use of these ^{90}Y-labeled antibodies in patients is the correlation between circulating blood levels in mice and the results of HPLC analysis. It appears that an important and possibly essential assay of label quality prior to patient administration is size exclusion HPLC analysis in which both the radiochemical purity and recovery are determined. Accordingly, only those preparations which display high (>95%) radiochemical purity and recovery will be considered for patient use.

In conclusion, we have demonstrated that previously reported methods may be adapted and others added to prepare DTPA-coupled antibodies labeled with ^{90}Y obtained from a radionuclide generator and that these preparations are of high radiochemical purity, are sterile and pyrogen free and suitable for human use.

NOTES

* Millipore Corporation, Bedford, MA.
† J.T. Baker Chemical Co., Phillipsburg, NJ.
‡ Amersham Corp., Arlington Heights, IL.
§ Fisher Scientific Co., Springfield, NJ.
¶ Staco Energy Products Co., Dayton, OH.
** Bio Rad Laboratories, Richmond, CA.
†† TGM Detector Inc., Waltham, MA.
‡‡ NEN-Dupont, Boston, MA.
§§ Canberra Industries, Inc., Meriden, CT.
¶¶ Charles River Supply, Wilmington, MA.

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