Binding of ^{99m}Technetium to Various Human Serum Albumins

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Abstract □ The percentage of ^{99m}technetium bound to Red Cross, Fraction V, and crystalline human serum albumin was investigated by dialysis and gel filtration. Any variation in the percentage bound seems to be a function of the technetium–iron–ascorbic acid complex not broken down rather than of any variation in the albumins.

Keyphrases □ ^{99m}Technetium—binding to human serum albumins, effect of variations in albumin, dialysis and gel filtration □ Albumins, various human—binding of ^{99m}technetium, effect of albumin purity, dialysis and gel filtration □ Dialysis—used to study binding of ^{99m}technetium to various human albumins □ Gel filtration—used to study binding of ^{99m}technetium to various human albumins

^{99m}Technetium-labeled serum albumin is used for scanning various organs and tissues; however, the fraction of ^{99m}technetium bound appears to vary substantially from preparation to preparation. In the work reported here, the possibility that some of this variation is associated with the purity of the albumin was checked. The binding of technetium to Red Cross serum albumin, Fraction V, and crystalline human serum albumin was investigated by dialysis and gel filtration.

METHODS

Materials—Normal serum albumin (human), salt poor[1], collected by the American National Red Cross, Washington, D. C., was used. This albumin is in 4.5% saline. Human albumin fraction V[2]; crystalline albumin, 100% pure[3]; and a generator kit[4] were obtained. Visking dialysis tubing[5] (27/32) was the dialyzing membrane. All other materials were either reagent grade or standard hospital pharmacy supplied.

Procedures—*Labeling of Human Serum Albumin*—The method recommended by Rajamani (1) was used. ^{99m}Technetium was eluted from the generator with normal saline, and 5 ml. of eluent was adjusted to pH 1.5 with 0.1 ml. 1 *N* hydrochloric acid solution. Ten milligrams of ascorbic acid and 10 mg. of ferric chloride hexahydrate were dissolved in this solution, and the pH was adjusted to 5.5 by the addition of 1.0 ml. 1 *N* sodium hydroxide. This was called Solution A.

Two and one-half milliliters of 10% dextrose was added to 4 ml. of 0.2 *M* acetate buffer, pH 5.2. Two milliliters of 25% normal serum albumin (human), salt poor, was added to this solution and stirred to give Solution B. Solution B was then added to solution A with stirring, and the pH was adjusted to 1.5 by the addition of 1.8 ml. 1 *N* hydrochloric acid. The solution was then incubated at room temperature for 15 min. and the pH was adjusted to 6.5 by adding 2.0 ml. 1 *N* sodium hydroxide.

For the experiments using crystalline and Fraction V human serum albumins, 25% solutions in 4.5% sodium chloride were used in place of the Red Cross albumin in the previous procedure. For the dialyzing solution and the developing solution for the gel filtration, a similar solution was used, except that the technetium solution was replaced by normal saline and the serum albumin solution was replaced by 4.5% sodium chloride.

[1] Processed by E. R. Squibb and Sons, Inc., New Brunswick, N. J.
[2] Armour Pharmaceutical Co., Kankakee, Ill.
[3] Schwartz/Mann, Orangeburg, N. Y.
[4] Pertgen-99m, Abbott Lab., North Chicago, Ill.
[5] Union Carbide, Chicago, Ill.

Table I—Percentage ^{99m}Technetium Bound to Human Serum Albumin

Human Serum Albumin	Preparation	Dialysis	Gel Filtration	B/A[a]
Red Cross	1	84.5(6) ± 1.5[b]	85.1(3)	0.10
Lot 01930	2	75.7(8) ± 3.5	81.4(3)	0.21
	3	76.5(8) ± 3.5	71.8(3)	0.19
Red Cross	1	83.5(5) ± 1.5	85.0(3)	0.10
Lot 01961	2	85.8(5) ± 0.5	90.4(3)	0.08
	3	90.9(5) ± 1.3	89.1(3)	0.11
Fraction V	1	95.9(5) ± 1.0	92.9(3)	0.06
	2	93.2(5) ± 0.6	93.5(3)	0.05
	3	92.0(6) ± 1.0	93.7(3)	0.04
Crystalline, 100%	1	87.3(6) ± 1.3	85.3(3)	0.10
	2	84.0(6) ± 1.3	77.2(3)	0.17

[a] B/A is the ratio of the iron–technetium–ascorbic acid complex to the technetium–albumin complex. [b] (±) indicates the range of values obtained.

Dialysis—The technetium-labeled human serum albumin solution was dialyzed against the solvent for 18–24 hr. at 22° in cells holding 5-ml. samples, using Visking dialysis tubing which had been pretreated for removal of glycerin and sulfides, as recommended by the manufacturer. Blanks were performed using equal concentrations of technetium-labeled human serum albumin on either side of the membrane so that any correction due to adsorption could be made.

Gel Filtration—Sephadex G25 was preswollen in the solvent, and a 50-ml. buret was used to give a column approximately 1.1 × 67.0 cm. A 0.5-ml. sample was placed on the column, and 2-ml. fractions were collected.

All solutions from the dialysis and gel filtration were counted using a well-counter[6].

RESULTS

The results expressed as percentage technetium bound are shown in Table I. Two batches of Red Cross albumin were compared with the more highly purified Fraction V and crystalline albumin. Both dialysis and gel filtration were carried out on each technetium–albumin preparation.

The numbers in parentheses indicate the number of samples measured on each preparation. All of the gel filtration figures were within 1% of the average indicated.

DISCUSSION

Figure 1 shows a typical gel filtration separation found with all of the systems investigated. The large peak (A) is the technetium bound to the albumin; the second peak (B), which contains no protein, seems to be of a technetium–iron–ascorbic acid complex on comparison with the separations reported by Richards and Atkins (2). The slower running peaks (C) are probably due to technetium in various states of oxidation (3). Peak C is very small compared to A and B. In the calculation of percentage bound, only the first peak was regarded as containing the bound material. In the early experiments with the Red Cross albumin, it was thought that peak B might be due to an impurity in the protein; however, subsequent measurements using the more highly purified materials showed this not to be so. The results, which show good agreement between gel filtration and dialysis, suggest that there is no advan-

[6] Abbott Logic Series model 111, Abbott Labs., North Chicago, Ill.

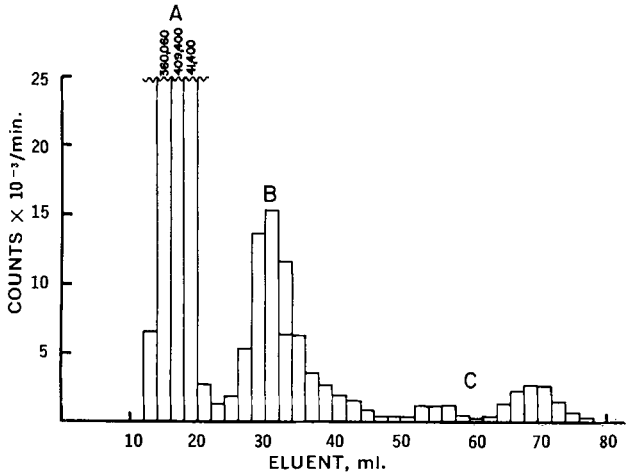

Figure 1—*Typical Sephadex G25 separation. Key: A, technetium bound to albumin; B, technetium–iron–ascorbic acid complex; and C, uncomplexed technetium in various states of oxidation.*

tage with the more highly purified protein fraction over the cheaper Red Cross material. However, the observations do show that there are significant variations in the fractions bound when apparently the same technique is continually used in radiopharmacy. According to the data in Table I, this variability seems to be associated with the technetium–iron complex. This complex is reported to be broken down when the pH of the complex–albumin mixture is lowered to 1.5 (2), as indicated in the *Methods* section. However, the observations reported here show the complex to be incompletely and variably broken down in the preparations.

The ratio of the second peak to the first peak (B/A) for the gel filtration is shown in Table I. It can be seen that when the percentage technetium bound is high, then the amount of complex broken down is also high, suggesting that a complete break of this complex is essential for maximal binding. In the first preparation using the second batch of Red Cross albumin, a dextrose solution containing 0.1% methylparaben was used rather than the plain dextrose and this caused the appearance of a second peak, smaller than the iron–technetium–ascorbic acid complex peak and running slightly slower than this peak; however, this did not substantially reduce the fraction technetium bound. This peak is possibly a second complex involving paraben. It is, of course, preferable not to use preservatives in intravenous injections.

Dowex 1 × 8 ion-exchange columns have been reported (2) to remove unbound technetium from solutions of the technetium–albumin complexes; such treatment, followed by a membrane filtration to give a sterile solution, seems to be necessary if near 100% binding is to be obtained. It also seems that adequate quality control over technetium–albumin products is obtained only if the percentage bound in every preparation is computed; the simplest technique to obtain this value is probably gel filtration. This can give better than 2% accuracy in 15–30 min. and can be performed in any radiopharmacy.

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