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Correlating Labeling Chemistry and In-Vitro Test Results with the Biological Behavior of Radiolabeled Proteins

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INTRODUCTION

A wide variety of iodination techniques as well as chelation with metallic radionuclides are currently utilized for radiolabeling of proteins, including monoclonal antibodies and their fragments. Whereas a number of proteins are relatively insensitive to mild chemical manipulation, many monoclonal antibody systems display varying degrees of altered biological behavior in particular following iodination using the common electrophilic substitution reactions. Different aspects of the in-vivo behavior are affected e.g., specificity of binding to in-vivo antigens, kinetics of blood and tissue uptake and clearance, rates and routes of excretion, etc. A number of these factors are related to the radionuclidic label itself and it is often necessary to consider these effects separately, since in most situations the radionuclide is the actual tracer and its own in-vivo distribution (when dissociated from the protein due to bond instability, ligand exchange, or catabolic processes if any, or if it overrides the targeting properties of the labeled protein) may happen to be quite

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different. Deleterious effects of labeling chemistry and purification schemes on the in-vivo behavior of the labeled protein must be clearly understood and minimized as much as possible. Further, it is necessary to establish a correspondence between the in-vitro test results and the observed in-vivo behavior in order to obtain predictable and reproducible data.

This article describes results from recent studies to investigate the effect of various labeling conditions on the functional integrity of a number of monoclonal antibodies. Factors affecting the binding to platelets of an antiplatelet monoclonal antibody following iodination (^{123}I, ^{125}I, ^{131}I) and chelation labeling with ^{111}In are addressed. Direct radiometal-labeling methods are discussed using the results from a study of ^{99m}Tc-Sn-HSA (human serum albumin) as an example. A brief description of the effect of degree of substitution, specific activity, chemical contaminants, and possible radiation damage on binding to platelets of the labeled antiplatelet antibody preparations is also included.

IODINATION

Techniques for iodinating proteins are well established and a variety of methods are currently utilized to obtain products labeled with ^{123}I, ^{125}I or ^{131}I. High specific activity nuclides enable one to label proteins at low iodine to protein substitution levels. Most procedures employ oxidizing agents such as Chloramine-T, Iodogen, lactoperoxidase (enzymatic reaction) etc., to render iodide into a reactive higher oxidation state species that generally iodinates tyrosine (most common), lysine or histidine residues on the protein molecule. Important factors that must be considered are the nature of the oxidant, ratio of oxidant to protein, reaction time, reaction volume, iodine to protein ratio, and the specific

activity of the labeled product. The above factors have to be optimized for individual proteins due to differences in their chemical sensitivity. The criteria of suitability of the labeled protein molecule are governed by the intended use of the product. Due to the sensitivity to chemical manipulations of the specific antigen- or receptor-binding sites of monoclonal antibodies, they are more susceptible to alterations in their biological behavior than are other conventional proteins such as albumin. Complete retention of immunological activity following iodination reactions is difficult to achieve in practice. However, by using very mild conditions often at the expense of low labeling yields, it is possible to obtain iodinated antibodies with satisfactorily high or even intact immunological activity.

Antiplatelet Antibody

Results on the iodination of an antiplatelet monoclonal antibody, 7E3 (Coller et al., 1983) using Chloramine-T are described in Table 1. It is apparent that by using higher amounts of the oxidant and/or longer reaction times, >90% labeling yields can be obtained. The percent labeling was based on trichloroacetic acid (TCA) precipitation or gel filtration using a G-25 Sephadex column. The following conditions provided the best product: 100 μg 7E3, 5 μg ChT, 2 min reaction time, 100-300 μl total volume, phosphate buffer, pH 7.0. Satisfactory labeling yields were also obtained using Iodogen. Reactions using Iodobeads, however, were much slower at low antibody concentrations and labeling yields were in the range of 10% (4 beads, 2 min) to 30% (4 beads, 90 min).

Binding to platelets of tracer doses (0.5-1 μg/ml blood) of minimally iodinated 7E3 was earlier shown to be ~75% (dog platelets), and ~90% (human

platelets) at saturation (60 min incubation with whole blood) (Srivastava et al., 1984a; Oster et al., 1985). The binding decreased with increasing I/7E3 ratios; there was a concommitant decrease in binding to the clots as well (formed by adding thrombin to whole blood). The data are described in Table 2. The Fab'_2 fragment of this antibody in one experiment displayed somewhat lower binding to platelets and to clots than the whole antibody. In-vivo blood clearance of I-7E3-platelets was not significantly affected up to an I/7E3 molar ratio of 5.

The stability with time of ^{131}I-7E3 is shown in Table 3. No significant decrease in the binding to platelets was observed up to 7 d storage at 4° in 0.2% HSA. At 15 d, however, the binding to platelets was lower even though the release of iodine label from 7E3 was not evident. When ^{131}I-7E3 was stored in the absence of HSA, much less binding resulted even at 2-7 d. The specific activity in these experiments was 30 ± 2 µCi ^{131}I/µg 7E3. In the case of ^{123}I, specific activities of up to 400 µCi/µg did not cause diminished binding (3 d storage in 0.2% HSA). It is obvious that some radiation damage results, especially when high specific activity preparations with ^{131}I are involved.

Anti-melanoma Antibody

A monoclonal antibody directed against a high molecular weight melanoma associated antigen (HMW-MAA), designated as 225.28s (Imai et al., 1981) was iodinated using chloramine-T, Iodogen, and lactoperoxidase methods. Radiochemical labeling yields were in the range of 30-80% depending upon the reaction conditions. Cell-binding assays (Figure 1) of the various ^{125}I-labeled preparations demonstrated reduced immune reactivity, and a significant difference in binding between products from Chloramine-T (43%), lactoperoxidase (30%) and Iodogen (24%) procedures (Srivastava et al., 1983). Whereas the sensitivity of

this antibody to iodination reactions and the relative merits of the three methods are obvious from the above assay, it is important to realize that generalizations for other antibody systems cannot be made since every antibody may display different sensitivity to same reagents and conditions. In the case of certain antibodies, iodination of tyrosine residues (most likely site) may lead to loss in immunoreactivity if tyrosine constitutes one of the antigen recognition sites.

Anti-colon Carcinoma Antibody

Results on the iodination of Fab'_2 fragment of the anti-colorectal carcinoma antibody, GA-733 (Herlyn et. al., 1984;1985) are shown in Table 4. This protein was also quite sensitive to iodination reactions. Using only very mild conditions was its immunoreactivity retained to an acceptable level. Iodogen was superior to Chloramine-T since it gave higher radiochemical yields while providing the labeled product with comparable immunoreactivity. When the reactions were allowed to proceed for a longer period (10 min), nearly quantitative iodination yield resulted. However, the cell binding of the labeled product dropped significantly.

Chemical and Radiochemical Purity of Iodine

The success of many protein iodination reactions depends on the chemical nature (oxidation state) of iodine in the radioiodine solutions. In addition, unwanted chemical contaminants that are invariably present due to processing chemistry and handling often exceed acceptable limits thus causing the labeling reactions to proceed very poorly or not at all in some instances. Added reducing agents (or those present as impurities) can very quickly consume the minute quantities (μg

amounts) of the oxidants used for converting I- ion to higher oxidation state iodine species that are required for effective protein iodination. Presence of iodate also causes reduced iodination yields. Even though the mechanisms of protein iodination reactions are not clearly understood, it appears that non-iodide forms of iodine if initially present cause greatly depressed labeling yields. The problems are somewhat more serious with iodine-123 solutions since the production, processing, and handling of this nuclide is quite variable. This results in non-uniform chemical and radiochemical purity of the available samples, particularly for the p,5n product. Recently developed HPLC methods (Srivastava et al., 1984b) allow for a quantitative analysis of iodine in various chemical forms, and often such quality control is helpful in explaining poor labeling yields and correlating them with the non-iodide species in the radioiodine solutions. Table 5 provides a summary of the HPLC data on iodine separations using a variety of reverse-phase columns and eluting solutions (Srivastava et al., 1984b). Figure 2 depicts a typical separation of iodide, iodate and periodate using a Lichrosorb RP-8 column.

Problems related to the source and purity of p,5n ^{123}I are exemplified from the data in Table 4. Using very mild and identical reaction conditions, the labeling yield of ^{123}I-GA-733 Fab'$_2$ was 45% in the case of BNL ^{123}I (95% iodide, no iodate) and 25% in the case of ^{123}I from another source (80% I^-, 15% IO_3^-, 5% miscellaneous species). The difference in immunoreactivity assay was even more dramatic: 57% cell binding versus 3% for the lower labeling yield product. The implication seems to be that in addition to causing depressed radiolabeling, the undesirable contaminants present in ^{123}I from one of the sources virtually destroyed the antigenic specificity of the GA-733 antibody fragment.

DIRECT LABELING WITH RADIOMETALS

Direct labeling of proteins with a number of metallic radionuclides has been carried out. Examples include ^{99m}Tc-labeled HSA, fibrinogen, and plasmin, etc. Various other radionuclides have also been employed, e.g., ^{51}Cr, ^{111}In, ^{113}In, ^{67}Ga, ^{68}Ga, and others. Proteins naturally consist of many functional groups that bind to metals with sufficiently high avidity and often give labeled products with high in-vitro and in-vivo stability. Labeling takes place through imidazole, carboxyl, sulfhydryl, and other electron donor groups on the proteins. Direct labeling of monoclonal antibodies has been less successful since many of these same functional groups are often responsible for the immunospecificity of the antibody which is compromised because of the attachment of the metallic nuclide.

A correlation between the labeling chemistry and in-vitro analytical results with the biological behavior of the labeled protein is exemplified by the ^{99m}Tc-Sn-HSA system. An optimized labeling procedure is as follows (Meinken et al., 1976; Klopper et al., 1979): (i) Prepare a fresh solution containing 0.21 M stannous chloride and 0.11 M DTPA in 0.3 M HCl; (ii) To a solution of 20-100 mg HSA (high purity, free of polymers) in 1 ml saline, add 0.5 ml of the above tin-DTPA solution. The resulting pH is ~2; (iii) Add the desired quantity of $^{99m}TcO_4^-$ in a volume of 0.5 to 2.5 ml saline, and mix for 30 min; (iv) Adjust pH to 6-6.5 with $NaHCO_3$ or NaOH. The possible components in ^{99m}Tc-HSA preparations are free pertechnetate, hydrolyzed or coprecipitated ^{99m}Tc (colloid), ^{99m}Tc chelate, ^{99m}Tc weakly bound to HSA, ^{99m}Tc-HSA aggregates and polymers, and the desired ^{99m}Tc-HSA monomer and dimer. Purification is best accomplished by using either polyacrylamide gel electrophoresis (PAGE), gel filtration using long (~100 cm) columns of G-100 Sephadex or A-0.5m Agarose (Table 6, Figure 3), or by HPLC

techniques. The ^{99m}Tc-HSA monomer and dimer (obtained in ~80% total yield using the above mentioned optimized procedure) give blood clearance curves in dogs that are similar to those obtained from commercial radioiodinated HSA (~80% remaining in blood at 2 hr). Blood clearance data of a number of commercial ^{99m}Tc-HSA kit preparations as well as the unpurified and purified fractions from column separations of the preparation from the procedure described above are shown in Table 7 and Figure 4. The in-vitro results (Table 6) correlate very well with the in-vivo blood clearance studies in dogs. The concentration ratio of ^{99m}Tc-HSA monomer to ^{131}-HSA in most organs was close to unity (Klopper et al., 1979). The initial rapid clearance of ^{99m}Tc activity encountered with most ^{99m}Tc-HSA preparations (Table 7, Figure 4) is associated with unbound or weakly bound ^{99m}Tc and/or colloidal ^{99m}Tc. The in-vitro test systems based on PAGE or high resolution gel filtration thus provide a very good correlation with the observed (or expected) in-vivo behavior of various labeled HSA preparations.

LABELING WITH RADIOMETALS VIA PROTEIN-CHELATING AGENT CONJUGATES

The "bifunctional chelating agent" approach of labeling proteins with metallic radionuclides was developed in 1974 (Sundberg et al., 1974) and later adapted and modified by many investigators (Krejcarek et al., 1976; Scheinberg et al., 1982; Hnatowich et al., 1983; Meares et al., 1984). There are several advantages to using this technique, in particular for labeling monoclonal antibodies. The most commonly used chelating agent is DTPA ("homobifunctional agent"). A number of other homobifunctional as well as heterobifunctional chelating agents are presently under development by various groups. These include EDTA analogs

(Scheinberg et al., 1982; Schmall et al., 1985), dithiosemicarbazone (Arano et al., 1984; Schmall et al., 1985), catechol (Schmall et al., 1985), desferrioxamine (Yokoyama et al., 1982), morphine and pyridine derivatives (Wang et al., 1985) nitrogen macrocycles (Meares et al., 1985) and metallothionein (Tolman et al., 1984). Many of these compounds may bind more specifically with certain metals (^{67}Cu, ^{97}Ru, ^{90}Y, etc.) and may permit, in addition to the current procedure of metal chelation following the conjugation of chelating agent to the antibody, the approach of forming the metal chelate first and then attaching it to the antibody (Cole et al., 1985; Schmall et al., 1985).

Monoclonal antibody-DTPA conjugates have successfully been labeled with various radiometals, including ^{111}In (Srivastava et al., 1983; 1984a; Oster et al., 1985; Fawwaz et al., 1985; Hnatowich et al., 1983; Scheinberg et al., 1982; Wang et al.,1984), ^{99m}Tc (Childs et al., 1985; Khaw et al., 1982), and ^{109}Pd (Fawwaz et al., 1984). Binding with other nuclides e.g., ^{67}Cu, is not stable in the presence of serum (or in-vivo) thus requiring the use of chelating agents other than DTPA (Cole et al., 1985; Meares, 1985). In this "chelation labeling" approach with monoclonal antibodies, several factors that affect radiochemical labeling yields and immunological properties must be carefully considered and optimized. These include (in the case of DTPA) the average number of DTPA groups per antibody molecule, source, purity and specific activity of the nuclide, effect of pH and competing ligands, serum stability, hydrolysis and redox reactions, and purification procedures.

Anti-melanoma Antibody

In a recent study (Wang et al., 1984) there was no significant difference in biodistribution in melanoma-bearing nude mice when ^{111}In-labeled antimelanoma

antibody (225.28s) was obtained using antibody-DTPA conjugates prepared by either the mixed anhydride method (Krejcarek et al., 1976) or the cyclic anhydride method (Eckelman et al., 1975; Hnatowich et al., 1983). The results are summarized in Table 8. This antibody was also successfully labeled with ^{109}Pd (Fawwaz et al., 1984) ^{65}Zn, and ^{67}Cu (Srivastava et al., 1985a) . The cell-binding assay showed that the average retention of immunoreactivity was 27, 41 and 15 percent, respectively, for ^{111}In, ^{109}Pd, and ^{65}Zn-labeled antibody. It should be noted that these variations are possibly due to the different chemistries involved in the labeling of the same antibody with different radiometals, the reasons for which are not entirely clear at this time. Data on the tumor uptake in mice of this antibody labeled with various nuclides are summarized in Table 9, along with the tumor-to-blood ratios. It is readily appreciated again that the in-vivo behavior is largely governed by the properties of the labeled product rather than totally by the antibody itself. Variations in labeling chemistry as well as the transformed in vivo properties of the metal-antibody complex (varying with the metal) would seem to account for these differences. Had the antibody suffered minimal alteration as a result of labeling, the tumor uptake and blood clearance for all radionuclidic labels would be the same. Obviously, this is not the case (Table 9). It appears that certain antibodies are more susceptible to labeling conditions than others. Labeling chemistries thus largely will have to be improved and optimized individually for particular antibodies and particular radionuclides.

Anti-platelet Antibody

The antiplatelet monoclonal antibody, 7E3, was labeled with ^{111}In using the DTPA cyclic anhydride conjugation method (Srivastava et al., 1984a; Oster et al.,

1985). Labeling yields ranged between 60 and 85%, depending on reaction conditions. The average number of conjugated DTPA groups per antibody molecule was kept at 5 or below. The specific activity of the preparations was 10-40 μCi ^{111}In/μg 7E3-DTPA at an average substitution level of 0.2 to 0.5 indium atoms per molecule. The source of ^{111}In was important. Trace metal contaminants commonly encountered in many commercial ^{111}In samples caused a wide variation in labeling yields. With increasing DTPA groups on the antibody, labeling yields were progressively higher. On the other hand, the binding to platelets of ^{111}In-DTPA-7E3 was reduced with increasing substitution level of DTPA on 7E3 (Table 10). The Fab'$_2$ fragment of this antibody conjugated with an average of 2.5 DTPA groups gave much reduced binding to platelets (Table 10). Increasing concentrations of antibody (>1 μg) per ml of blood (during in-vitro incubation for platelet labeling) resulted in faster blood clearance and diminished uptake in in-vivo thrombi in dogs (Srivastava et al., 1985b).

CONCLUSIONS

Highly specific monoclonal antibodies have recently become available for research and clinical applications in nuclear medicine. These antibodies possess enormous potential for use as agents to selectively transport diagnostic as well as therapeutic amounts of radionuclides to target antigens in vivo, in particular for tumor imaging and therapy. While the principle is straightforward, successful translation of this concept into practice has encountered numerous problems. The techniques and the chemistry involved in labeling antibodies with iodine or metallic radionuclides are of paramount importance. Whereas general protein

radiolabeling methods are useful and applicable to antibodies, immunological properties of the antibodies are often compromised resulting in reduced in-vivo specificity for the target antigens. A number of techniques have been and are being developed to attach radionuclides without causing undesirable alterations in the biological properties of monoclonal antibodies. The bifunctional chelating agent approach shows the most promise; however, development of agents other than DTPA (most commonly used) will be necessary for widespread usefulness of this technique. The effects of labeling chemistry on the in-vivo behavior of proteins, in particular of antibodies, must be clearly understood and resolved. A correspondence between in-vitro tests and the in-vivo performance of the labeled products must be established in order to obtain predictable and reproducible results.

Representative results briefly described in this chapter, and the experience of various investigators including ourselves, demonstrate that while some generalizations are possible and others may result from future investigations, it will be necessary to carefully optimize labeling and purification procedures for individual antibodies and particular radionuclides in order to achieve maximum efficacy of the labeled monoclonal antibody technique for imaging or therapy applications.

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FIGURE CAPTIONS

Figure 1. Comparison of in vitro cell binding efficiency of three different preparations of antimelanoma antibody labeled with iodine-125 using chloramine-T (ChT), Iodogen, and lactoperoxidase (LPO). 1×10^5 human COLO 38 melanoma cells were incubated with decreasing concentrations of radiolabeled antibody in a 96-well pvc micro-titer plate. Percent of radioactivity bound to cells was determined after extensive cell washing.

Figure 2. A typical HPLC separation of various chemical forms of iodine. The sample shown was prepared by mixing the individual solutions immediately prior to use. (Upon aging the iodide gets quantitatively oxidized by periodate to produce iodate.) Column: Lichrosorb RP-8, 4.6 x 250 mm. Eluting solution: 5×10^{-2} M PO_4^{3-} and 2×10^{-3} M Bu_4NOH in 20% methanol; pH 7.0. Flow rate, 1 ml per min, temp. 22°.

Figure 3. Fractionation of ^{99m}Tc-HSA preparation (BNL, DTPA procedure) on Agarose A-0.5m column (0.9 x 100 cm). Open squares: radioactivity; open circles: transmission at 280 nm. 1, void volume fraction (polymer or aggregate); 2, Trimer; 3, Dimer; 4, Monomer.

Figure 4. Blood clearance curves of various labeled albumin preparations in dogs (n = 4-10) compared to monomeric (purified) I-131 albumin. Curves 1, 3-5, 9 - BNL Tc-99m-albumin: 1, monomer; 3, dimer; 4, unpurified; 5, trimer; 9, polymer. Curve 2: commercial I-131-albumin, used as supplied. Curves 6, 7, and 8: Tc-99m-albumin prepared using various commercial kits, not purified after labeling.

TABLE 1. Iodination of Antiplatelet Antibody, 7E3 (100 μg), Using Chloramine-T Under Various Conditions[a]

Nuclide	ChT, μg	Reaction Time, min	% Labeling Yield (TCA, G-25)
^{123}I	15*	10	78
	5	2	70
	1.5*	10	47
^{125}I	15*	10	92
^{131}I	1.5	2	28
	1.5	30	45
	5	2	75
	5	10	85

[a]Each number is average of 3 experiments. Reaction volume 200 ± 100 μl, phosphate buffer, pH 7.0
Ratio of I/7E3 = 1.0 except * = 2.

TABLE 2. In-vitro Binding to Dog Platelets[a] and to Clots of ^{131}I-7E3 as a Function of I/7E3 Ratio

Ratio I/7E3	Labeling yield (TCA), %	Percent Binding	
		Platelets	Clot
0.5	70	78	70
1.0	75	75	68
2	80	71	65
5	85	60	50
10	86	50	40
22	90	12	10
1.0 (Fab'2)	84	67	55

[a] In whole blood, 60 min incubation.

TABLE 3. Stability with Time of ^{131}I-7E3[a]

Time, Days	Bound ^{131}I, % (TCA)	Binding to Platelets, %
0	78	80
7	75	72
15	74	55

[a]Ratio I/7E3 = 1.0; sp. activity 30 ± 2 μCi/μg. Stored at 4° in 0.2% HSA, Tris/saline buffer, pH 7.

TABLE 4. Effect of Iodination Conditions on GA-733 Fab'$_2$[a]

Reaction Conditions				
Nuclide	Oxidant	Reaction Time, Min	Labeling Yield, %	Immunoreactivity (Cell-Binding Assay)
^{131}I	Chloramine T	1	37	56.5
	(5 μg)	10	92	42
	Iodogen	1	61	63
	(7.5 μg)	10	94	35.5
^{123}I (p,5n)	Chloramine T	1	45	57[b]
	(5 μg)	1	25	3[c]

[a]No difference in labeling yields or immunoreactivity with iodine to antibody ratios of 0.5-2. Average sp. act.: 5 μCi/μg(^{131}I); 40 μCi/μg (^{123}I).
[b]BNL produced.
[c]Another source.

TABLE 5. Analysis of Various Radioiodine Species (k' values)[a] by C_2, C_8 and C_{18} Reverse-Phase High Performance Liquid Chromatography[b]

Species	100% Aqueous			10% Acetonitrile			40% Acetonitrile		
	C_2	C_8	C_{18}	C_2	C_8	C_{18}	C_2	C_8	C_{18}
IO_3^-	0.09	0	0	0	0	0	0	0	0
I^-	1.55	4.86	3.29	0.73	1.21	0.43	0.09	0	0
IO_4^-	11.2	-	R[c]	3.55	5.36	6.57	1.36	-	0
CH_3I	1.64	-	3.71	1.55	4.14	2.86	1.0	1.57	1.0
CHI_3	R	-	R	R	R	R	3.0	10.7	3.71

[a] $k' = \dfrac{\text{elution volume (Ve) - column volume (Vo)}}{\text{Vo}}$

[b] Elution buffer was a 0.05 M pH 7 phosphate solution containing the stated amount of aqueous or organic phase and 0.002 M tetrabutylammonium hydroxide.

[c] R, retained.

TABLE 6. In Vitro Data for ^{131}I-HSA and ^{99m}Tc-HSA Preparations[1]

Formulation, ^{99m}Tc-Albumin[2]	Percent activity bound to albumin				
	Electrophoresis		Gel Filtration[3]		
	Cellulose Acetate	Polyacrylamide Gel (PAGE)	Sephadex G-25	Sephadex G-100	Biogel A-0.5m
DTPA procedure	77	35 (D); 44 (M)	99[a] 93[b]	42 (P); 46 (M)[c]	40 (D); 36 (M)[b]
Citrate procedure	83	-	-	-	13 (D); 64 (M)[b]
DTPA procedure using crystalline HSA	86	43 (M); 39 (D)	-	36 (P); 44 (M)[b]	-
DTPA procedure using canine albumin	75	-	-	-	-
Commercial electrolytic Kit	64	-	58[a]	-	-
Commercial ^{131}I-HSA[4]	85	80 (M); 5 (D)	95[a]	-	4 (D); 83 (M)[b]

1. Average of 4-10 experiments for each data point.
2. Unless otherwise mentioned, 25% HSA solution (Hyland) was used. Pertechnetate was not detected in any of the preparations. Unbound activity (balance) represents reduced technetium (chelated, weakly HSA-bound or colloidal).
3. Column size: a, 0.9 x 35 cm; b, 0.9 x 100 cm; c, 3.0 x 100 cm. Supporting bed ~90% of column height. Fractions as follows: P, polymer; D, dimer; M, monomer.
4. 3% free iodide by paper chromatography (85% MeOH).

TABLE 7. Blood Clearance of Various Labeled Albumins in Dogs[a]

	Percent Remaining in Blood									
	A	1	2	3	4	5	6	7	8	9
Time (min)	^{131}I-HSA Monomer	^{99m}Tc-HSA Monomer (BNL)	^{131}I-HSA Unpurified	^{99m}Tc-HSA Dimer (BNL)	^{99m}Tc Unpurified (BNL)	^{99m}Tc-HSA Trimer (BNL)	^{99m}Tc-HSA Commercial Kit, Company X	^{99m}Tc-HSA Commercial Kit, Company Y	^{99m}Tc-HSA Commercial Kit, Company Z	^{99m}Tc-HSA Polymer (BNL)
1	92.4	97.7	96.6	96.5	100	97.3	99.2	97.3	102.5	75.6
5	96.9	96.8	96.8	94.4	98.7	92.0	95.2	84.0	87.3	33.1
15	95.3	93.1	93.2	88.2	91.6	85.0	86.4	72.6	76.1	14.2
30	89.9	89.3	86.1	81.1	78.0	78.5	79.8	64.7	65.0	11.4
60	86.0	84.0	81.4	74.3	76.4	74.7	68.4	55.6	52.0	9.4
90	87.6	81.4	82.0	71.4	70.9	68.1	68.7	50.6	45.8	8.4
120	84.9	82.1	78.4	71.2	67.1	66.1	64.4	45.9	41.0	7.4
Fast Component										
T 1/2 (min)	14	14	18.7	14	34	7.5	21.3	7.0	10.3	2.5
% of Total	9	12	15.6	22	31	16	30	37	33.5	88
% Excretion in urine (2 hr)	0.02	2	-	12	21.2	5.3	-	21.5	16.3	12.2

[a]Numbers of various preparations correspond to curves in Figure 4. Each data point is an average of 3-10 animal experiments.

TABLE 8. Tissue Distribution in Melanoma Bearing Mice (% Injected Dose per g.) of ^{111}In-DTPA-anti-HMW-MAA Monoclonal Antibody, 225.28s, Prepared Using two Methods of Chelate Conjugation (96 hr Post Injection)

Tissue	Method	
	Mixed Anhydride	Cyclic Anhydride
Blood	2.1 ± 0.5	3.3 ± 0.8
Tumor	12.6 ± 2.9	16.1 ± 4.1
Muscle	0.5 ± 0.1	0.4 ± 0.1
Bone	1.9 ± 0.5	1.6 ± 0.2
Liver	9.1 ± 0.3	7.7 ± 1.0
Spleen	4.3 ± 0.2	3.8 ± 0.2
Kidney	9.2 ± 0.8	7.7 ± 0.6
Lung	1.9 ± 0.5	2.7 ± 0.5

TABLE 9. Tumor Uptake and Tumor to Blood Ratios in COLO 38 Melanoma Bearing Nude Mice of Labeled Antimelanoma Monoclonal Antibody, 225.28s, 4 d Following Injection

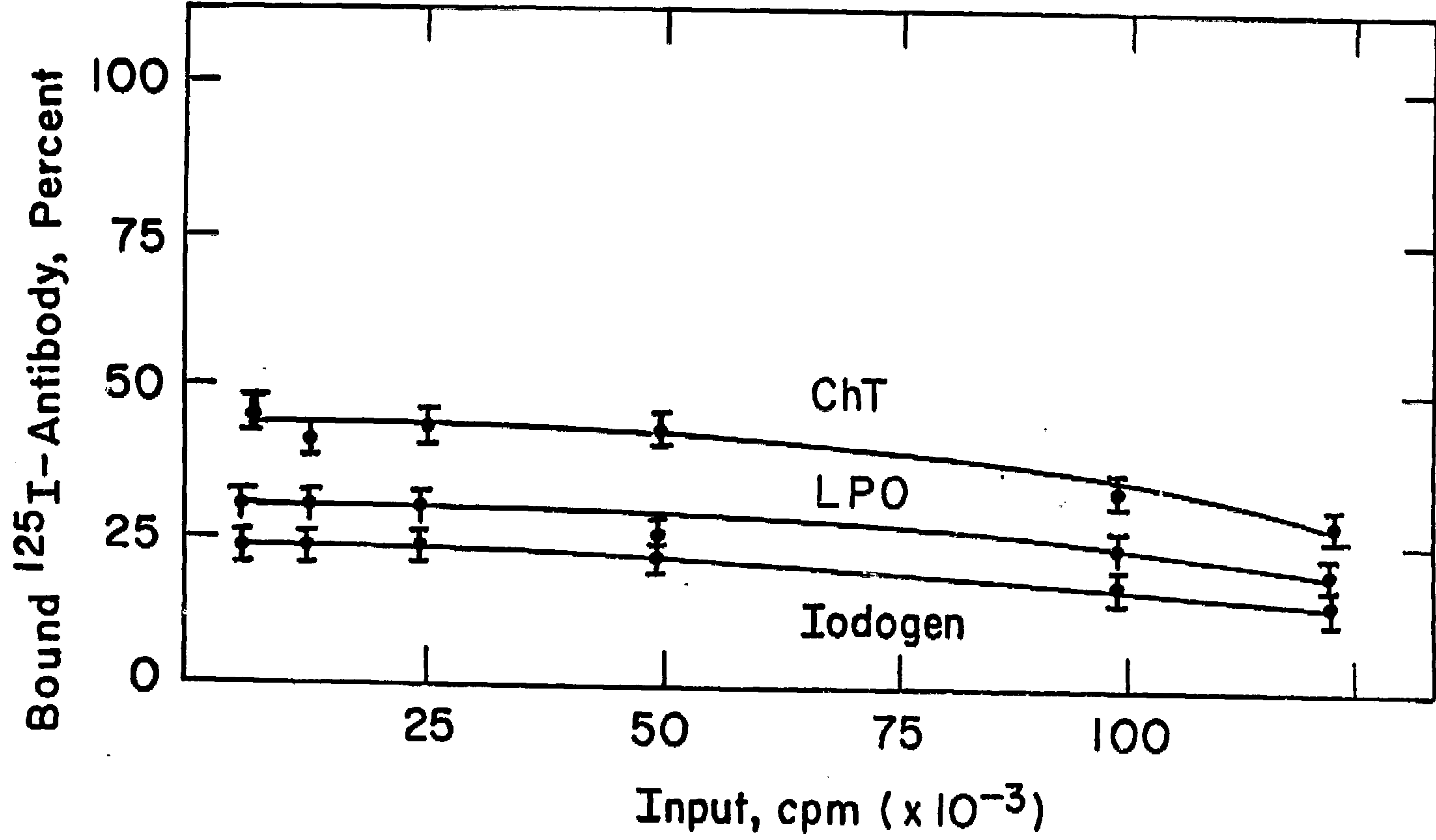
Radionuclide	Tumor/Blood Ratio	% ID/g in Tumor
^{111}In	4.9	16.1 ± 4.1
$^{109}Pd^{a}$	63	18.9 ± 4.4
^{65}Zn	15	3.0 ± 0.9
^{125}I	5.6	2.3 ± 0.1

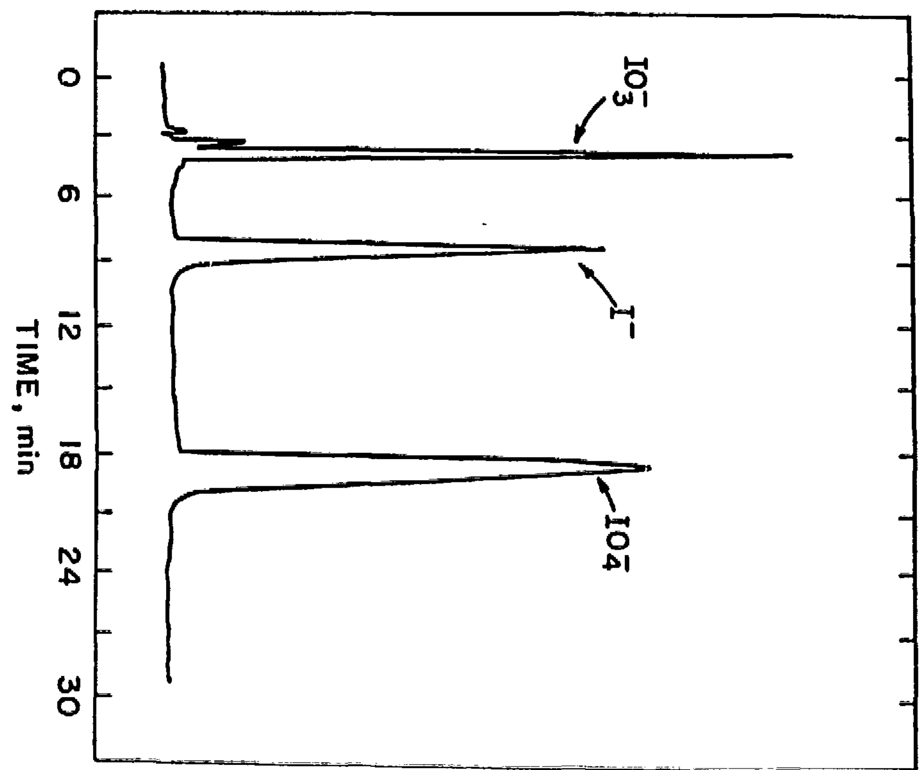
[a] 48 hr after injection.

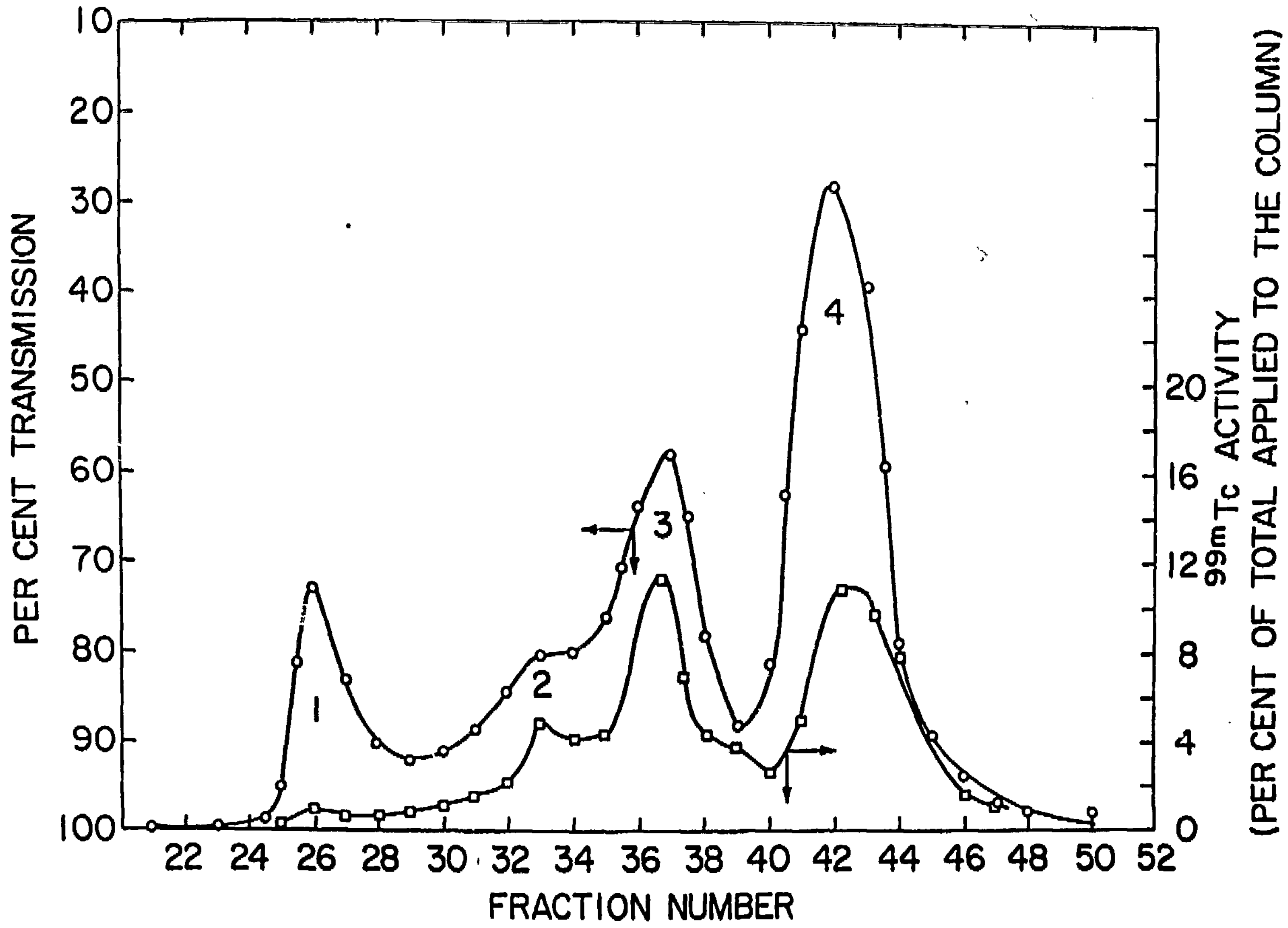
TABLE 10. Binding to Platelets of ^{111}In-7E3 as a Function of DTPA/7E3 Ratio[a]

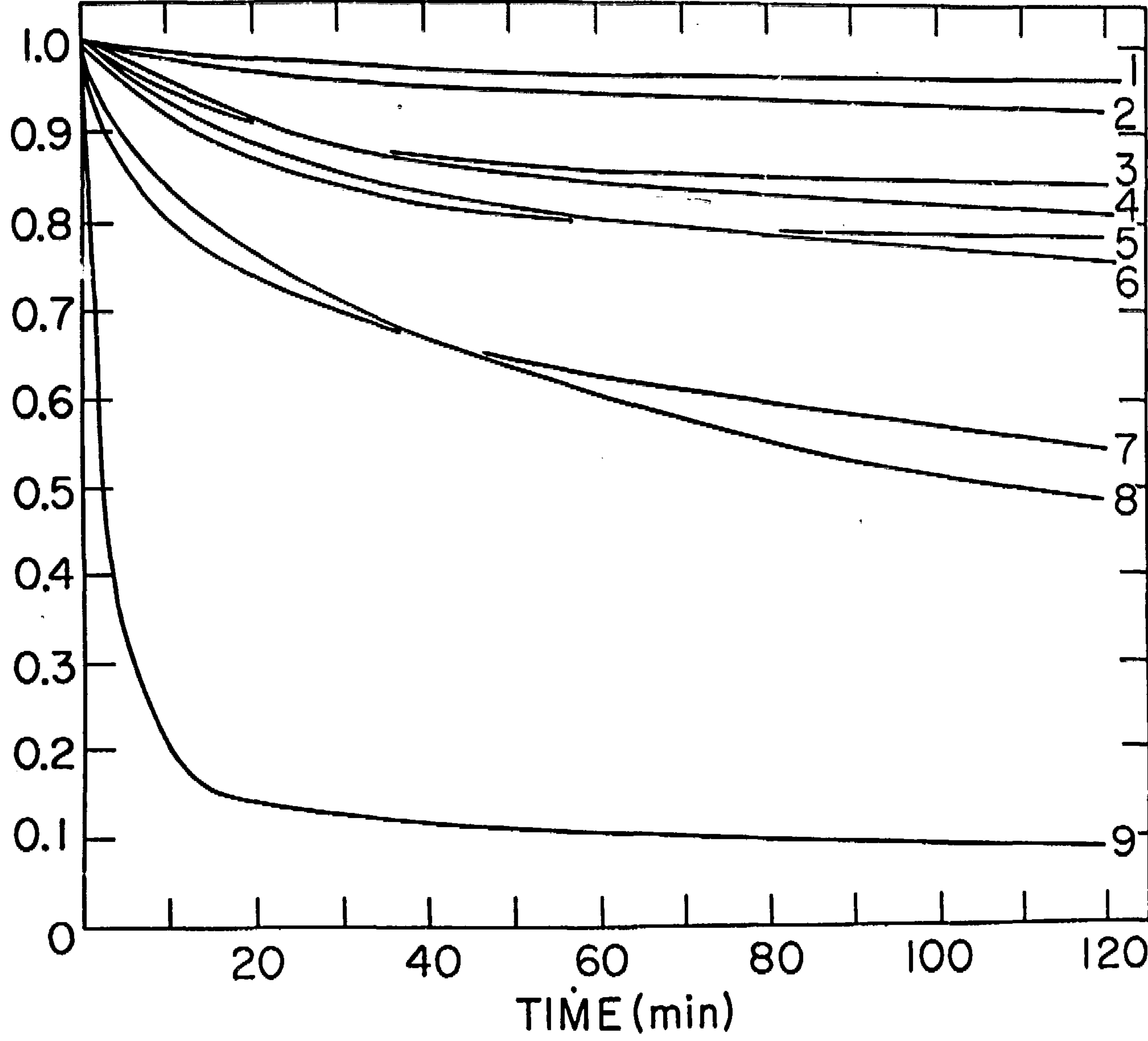
Ratio DTPA/7E3	Labeling Yield, %	Binding to Platelets, %
0.9	78	72
1.5	80	68
5.3	85	59
2.5 (Fab'_2)	82	20

[a]In vitro incubation with whole dog blood, 60 min.


Bound ^{125}I-Antibody, Percent
100
75
50
25
0
ChT
LPO
Iodogen
25
50
75
100
Input, cpm (x 10^{-3})


ABSORBANCE, 225 nm
TIME, min
0
6
12
18
24
30
IO_3^-
I^-
IO_4^-


PER CENT TRANSMISSION
10 20 30 40 50 60 70 80 90 100
FRACTION NUMBER
22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52
1
2
3
4
0 4 8 12 16 20
99m Tc ACTIVITY
(PER CENT OF TOTAL APPLIED TO THE COLUMN)


ACTIVITY RATIO, LABELED ALBUMIN:
MONOMERIC ^{131}I-ALBUMIN
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
20
40
60
80
100
120
TIME (min)
1
2
3
4
5
6
7
8
9