# 2. SUMMARY REPORTS OF PARTICIPATING COUNTRIES



## 2.1. ARGENTINA

Research contract No. 6282/RB

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| Title of the Project | : | Development of Radiolabelling Techniques of Anti-CEA Monoclonal Antibody |
| Chief Scientific Investigator | : | S.G. de Castiglia<br>Comisión Nacional de Energía Atomica,<br>Centro Atómico Ezeiza, Buenos Aires |

### 2.1.1. Introduction

Different radionuclides have been tried for radiolabelling of antibodies in order to localize small tumours [1]. $^{99}Tc^{m}$ is the most popular radionuclide for clinical imaging because it has ideal nuclear properties, i.e. a single photon energy of 140 KeV, a half-life of 6 h and it is readily available from a $^{99}Mo$-$^{99}Tc^{m}$ generator. Antibodies labelled with $^{99}Tc^{m}$ should be useful in tumour detection if localization and blood clearance are rapid enough to take advantage of the short half-life. There are different routes for labelling antibodies with $^{99}Tc^{m}$ directly, that is reacting endogenous sulfhydryl groups generated within the antibody [2] and indirectly, either before or after attachment of an exogenous chelator [3–4] to the antibody to bind reduced $^{99}Tc^{m}$. Recently, more attention has been paid to the direct labelling method due to the possibility of an "instant kit" formulation. In most cases the direct method is a combination of two sequential steps: the reduction of disulfide groups in the antibody and the use of a ligand capable of transferring the reduced technetium to the sulfhydryl groups of the protein [5–7]. Normally a purification step is necessary in order to remove the excess of reductant. Sykes et al. [8] have developed a photoactivation method based on irradiation of antibodies with ultraviolet light in order to reduce disulphide bonds. The advantage of this method is that no purification step is needed.

In indirect methods, technetium is coordinated by a synthetic chelating agent which may be conjugated to the immunoglobulin either before, or after, the radiolabelling process. The hydrazinonicotinamide system developed by Abrams and co-workers [9] has been successfully used for preparation of specifically labelled proteins and peptides with high specific activities. The chelator is covalently attached and labelling is accomplished by transchelation from $^{99}Tc^{m}$ glucoheptonate (GH) and $^{99}Tc^{m}$ tyrosine to produce $^{99}Tc^{m}$ products.

Recently, Winnard et al. [10] have developed a simple synthesis of the N-hydroxysuccinimide derivative of $MAG_3$ (NHS-$MAG_3$) in which a different protecting group (acetyl) from $MAG_3$ has been used. labelling with $^{99}Tc^{m}$ is achieved at room temperature by transchelation from $^{99}Tc^{m}$ glucoheptonate.

The purpose of this work was to label monoclonal and polyclonal antibodies with $^{99}Tc^{m}$ such as the ior-CEA-1 antibody and polyclonal IgG using a direct method, to check the radiochemical and biological behavior of labelled products, to prepare it under sterile and apyrogenic conditions as a lyophilized kit and to employ it in clinical trials. In addition, a photoactivation method was used to label polyclonal IgG with $^{99}Tc^{m}$ and to compare with the established method using mercaptoethanol (2-ME) as the reducing agent. Finally polyclonal IgG was labelled using an indirect method in which a chelator was covalently attached to the protein and the $^{99}Tc^{m}$ added as a glucoheptonate complex. The properties of $^{99}Tc^{m}$ when labelled with monoclonal and polyclonal antibodies by different methods were assessed by in vitro and in vivo studies.

### 2.1.2. Materials and methods

#### *2.1.2.1. Direct labelling of ior-CEA-1 antibody*

***Labelling and quality control***

Ior-CEA-1 monoclonal antibody (MoAb), produced by the Centro de Inmunologia de la Habana, Cuba, which recognizes the carcinoembryonic antigen (CEA), was prepared at a concentration of 10 mg/mL in 0.1 M PBS, pH7.0 and labelled with $^{99}Tc^{m}$ using a direct method in order to be used in immunoscintigraphy. Monoclonal antibody purity was assessed before and after labelling by polyacrylamide gel electrophoresis (SDS-PAGE) and size exclusion HPLC. 2-ME was used as a reducing agent for the protein and a methylenediphosphonate (MDP) kit and $^{99}Tc^{m}$ for the labelling step. Several molar ratios (2-ME:MoAb) were tested and 1000:1 ratio was used to reduce the protein for 30 min. The solution was then purified on a Sephadex G50 column (Pharmacia, Uppsala, Sweden) and eluted with nitrogen purged phosphate buffer 10 mM pH6.0. Fractions were collected and measured at 280 nm and concentration fractions higher than 0.5 mg/mL were pooled. An aliquot (40 $\mu$L) of the MDP kit [containing 1.0 mg of MDP and 68 $\mu$g of $SnF_2$ per mL after reconstitution] was added followed by 187.5 MBq $^{99}Tc^{m}O_4$. Labelling efficiency was measured by instant thin layer chromatography (ITLC) developed in MEK and saline. In addition, the radiochemical purity of the labelled antibody was analyzed by size exclusion HPLC using a Protein Pak 300 (Waters) column with 0.1M phosphate buffer eluent pH7.0 at a flow rate of 1 mL/min. Recovery of the radioactivity was routinely determined.

Labelled antibody was tested for stability towards cysteine. A solution of cysteine (27 mg/mL, 100 mM) in PBS was prepared and diluted to a cysteine concentration of 0.1, 1, 5, 10, 32 and 64 mM. Each cysteine solution was added to a tube followed by a fixed volume of the labelled protein and tubes were incubated at 37°C for one hour. The cysteine: antibody molar ratios ranged from 700:1 at 100 mM of cysteine to zero in the absence of cysteine. At the end of the incubation each solution was spotted on ITLC and developed in saline and MEK.

Biodistributions in normal Balb/c mice were performed at 4 h after injection of 750 kBq of labelled antibody.

A competitive binding assay comparing the reduced antibody with unreduced antibody was carried out using CEA as antigen. Increasing amounts of native and reduced antibody were added to CEA coated tubes followed by a fixed volume of labelled antibody.

In addition an ELISA was performed comparing the binding of the reduced and native antibody with the purified antigen. Microwell plates were coated with the antigen and saturated with PBS-5% BSA. Serial dilutions of native and reduced antibody were added to each well and the binding was revealed with a rabbit antimouse antibody labelled with peroxidase. After washing the plates, the enzymatic reaction was developed with a chromogenic substrate and stopped by addition of $H_2SO_4$. The optical density reading was at 492 nm.

#### *2.1.2.2. Lyophilized kit of IgG*

***Labelling and quality control of IgG***

The protein reduction step was achieved following the antibody reduction protocol and two molar ratios of 2-ME and protein were tried, 500:1 and 1000:1. The concentration of unreduced protein was 20 mg/mL. Lyophilized kits of reduced IgG (1 mg) were performed with the addition of 40 $\mu$L of a fresh MDP kit (1.0 mg of MDP and 68 $\mu$g of $SnF_2$ per mL) per mg of reduced protein. At the end of the experiments, the kit was labelled with 562.5 MBq of $^{99}Tc^{m}$ and labelling efficiency was measured by chromatographic methods: size exclusion HPLC and ITLC developed in MEK and saline. According to the obtained results, a bulk reduction of protein (50 mg) was performed at a

500:1 molar ratio. After purification aliquots containing 1 mg of reduced purified protein and MDP/$SnF_2$ were lyophilized. The composition of the kit was: 1 mg of reduced protein, 3 $\mu$g of $SnF_2$, 40 $\mu$g of MDP and saccharose as lyoprotectant. The kit was reconstituted with 2 mL of saline and the required activity of $^{99}Tc^m$ was added.

***Biological studies***

An animal model was developed injecting 40 $\mu$L of turpentine in the posterior left thigh of Balb/c mice weighing approximately 25 gm. They were left for 48 h in normal conditions to develop the inflammation foci. Mice were injected with 7.5 MBq of IgG labelled with $^{99}Tc^m$ and images were taken at 4 and 24 h with a gamma camera equipped with a medium energy collimator and 70 000 counts were preset using a 256 × 256 matrix. Biodistributions in normal Balb/c mice and in mice bearing a promoted inflammation foci were carried out at 4 and 24 h post injection of 1.875 MBq. of labelled IgG. The total injected dose was calculated by measuring syringes before and after injecting each animal. Five animals from each group were sacrificed by cervical dislocation at 4 and 24 h after injection. Samples of blood were taken and organs and tissues of interest were dissected, rinsed, dried and placed into pre-weighed tubes. The activity of all samples was counted together with appropriate dilutions of the labelled IgG and results were expressed as % ID/gm.

Stability towards storage was examined performing labelling tests at 15 days, 1, 2, 3, 4, 6 months after lyophilization. SDS PAGE was performed at the beginning and at the end of the experiment.

*2.1.2.3. Lyophilized kit of ior-CEA-1 antibody*

Similar experiments were done in order to obtain a lyophilized kit of reduced antibody. In this case a molar ratio of 800:1 was used and a concentration of unreduced antibody of 10–15 mg/mL. Within the quality assurance procedure the radiochemical purity, stability, sterility, apyrogenicity, immunoreactivity and biodistribution in normal Balb/c mice were studied.

*2.1.2.4. Labelling of IgG by photoactivation*

***Labelling and quality control of photoactivated IgG***

Experiments were carried out with this method to find the formulation of optimal binding of $^{99}Tc^m$ to polyclonal IgG. In addition animal studies in normal mice and in mice bearing a promoted inflammation foci were performed.

A photochemical reactor Rayonet RMR 3000 with eight UV lamps was used. A typical labelling procedure for IgG was as follows: 100 $\mu$L of a solution of IgG in PBS (5 mg/mL) was injected into a 10 mL vial and purged with nitrogen. A fresh solution containing stannous fluoride (18/100 $\mu$L) and MDP was purged with nitrogen for 10 min and 100 $\mu$L of this solution was injected into the vial containing the protein. The vial was irradiated for 20 min in a photochemical reactor. Sodium [$^{99}Tc^m$] pertechnetate was added to the vial and the solution was incubated for 30 min at RT. The labelled product was analyzed by size-exclusion HPLC and ITLC in saline and MEK systems.

Different parameters of the labelling reaction were evaluated such as: a) amount of tin, b) protein concentration, c) effect of storage on labelling efficiency. Labelling experiments were carried out to assess the binding of $^{99}Tc^m$ to photoactivated IgG with different amounts of tin and to photoactivated IgG of different concentrations and the same amount of tin. Irradiation time and incubation time were kept constant in all the experiments. A bulk irradiation was performed in a vial containing 10 mg of IgG (10 mg/mL) and 2 mL of a solution of MDP/tin (43 $\mu$g of tin/mg of protein). One aliquot was immediately labelled with the radionuclide and the other aliquots were stored at -20°C and -70 °C and labelled at different time intervals.

Biodistribution studies were carried out in Balb/c mice and in mice with a sterile turpentine induced inflammation in the left thigh. Both groups were injected intravenously in the tail vein with 100 $\mu$L of photoactivated IgG (20 $\mu$g) and results were obtained according to the protocol described above. In addition a group of four normal Balb/c mice was injected intravenously with 100 $\mu$L of labelled IgG and each mouse was counted within a well-type ionization dose calibrator at 1 h intervals over the next 4–5 h. Loss of whole body radioactivity not accounted for by physical decay was assumed to be due to urinary excretion.

Transchelation to cysteine was performed at cysteine concentration ranging from 1000 $\mu$M to 0 $\mu$M. A comparison was performed between the labelled photoactivated IgG and the labelled IgG via the 2-ME reduction method.

### *2.1.2.5. Indirect labelling of IgG*

***Conjugation, labelling and quality control***

Human polyclonal IgG (Sandoz) was conjugated with the NHS-$MAG_3$ chelator (a gift from Dr. Hnatowich, USA) according to the following protocol: IgG at a concentration of 10 mg/mL in PBS 10mM pH7.4 was conjugated at pH8.5 in bicarbonate buffer. A solution of NHS-$MAG_3$ (10 mg/mL) in dry DMSO was added at a molar ratio of 10:1 $MAG_3$:IgG. The mixture was incubated at room temperature for 30 min and purified on a 0.7 × 20 cm column of Sephadex G-50 using 0.2M ammonium acetate buffer, pH5.2. Protein fractions were collected and concentrations measured by UV absorbance at 280 nm. The protein was radiolabelled with $^{99}Tc^m$ using glucoheptonate as transchelator. Pertechnetate was added to provide about 370–1850 MBq/mg of IgG. A freshly prepared solution (40 mg/mL) of sodium glucoheptonate (GH) in 1M bicarbonate, 0.25M ammonium acetate and 0.175 M ammonium buffer, pH8.5–9.0 was added in a sufficient volume to provide a final glucoheptonate concentration of 5.0–5.5 mg/mL. Finally, stannous ion was added from a fresh 0.5 mg/mL solution in ClH (1 $\mu$g of Sn per 5–200 $\mu$g of IgG and 1.875–18.75 MBq $^{99}Tc^m$). The labelling solution was incubated at RT for 30–40 min and purified using a centrifugal filter (Centricon 30).

The labelled product was evaluated for radiochemical purity by high performance liquid chromatography analysis (HPLC) using a size exclusion column Protein Pak SW300 eluted with PBS 10 mM. Recovery was routinely determined. The product was also checked with ITLC in MEK and saline systems.

Labelled IgG was incubated at room temperature for 24 h. At various time points, aliquots of the sample were removed and analysed by HPLC and ITLC.

A cysteine challenge was performed with the labelled product. A fresh cysteine solution in phosphate buffer 0.4M, pH7.0 was prepared (0.082M). To 12 $\mu$L of five diluted cysteine solutions, 90 $\mu$L of the labelled protein (1.3 uM) was added. Final concentrations of cysteine were 1000, 500, 50, 5 and 0.5 $\mu$M. The highest molar ratio of cysteine to antibody was 908:1. After incubation at 37°C for one hour the percent of dissociation was measured by ITLC in saline.

Biodistribution studies were carried out in Balb/c mice and in mice with a sterile turpentine induced inflammation in the left thigh. Both groups were injected intravenously in the tail vein with 100 $\mu$L of technetium-99m labelled to IgG via the bifunctional chelator NHS-$MAG_3$ (20 $\mu$g) and results were obtained according to the protocol described above.

In addition a group of four normal Balb/c mice was injected intravenously with 100 $\mu$L of labelled IgG and each mouse was counted within a well type ionization dose calibrator at 1 h intervals over the next 4–5 h.

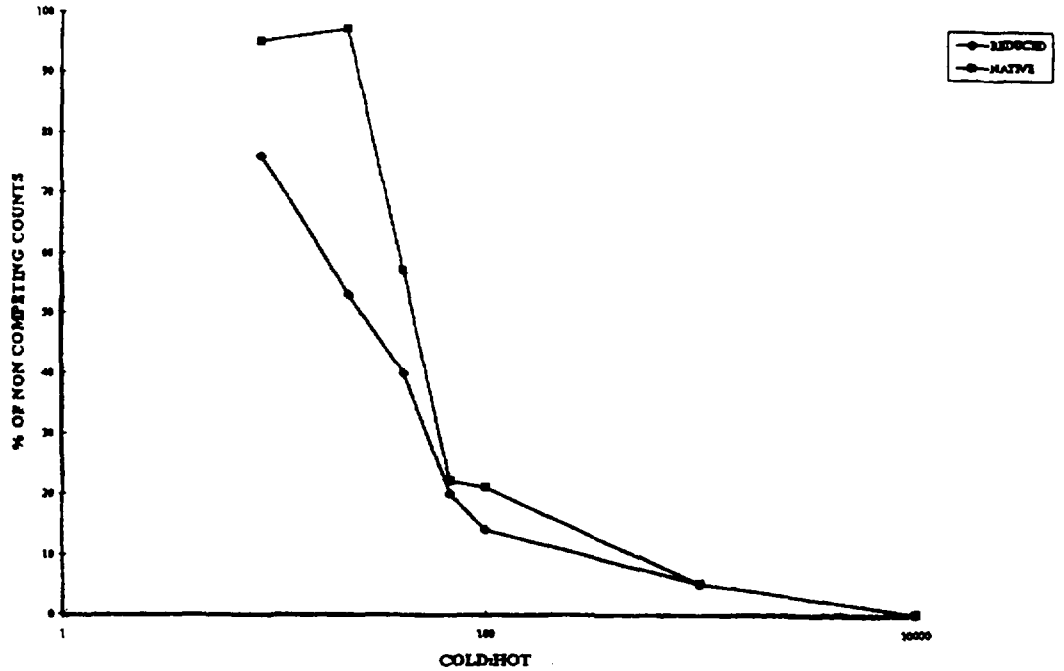


*FIG. 1. Competitive binding assay comparing the reduced antibody with unreduced antibody.*

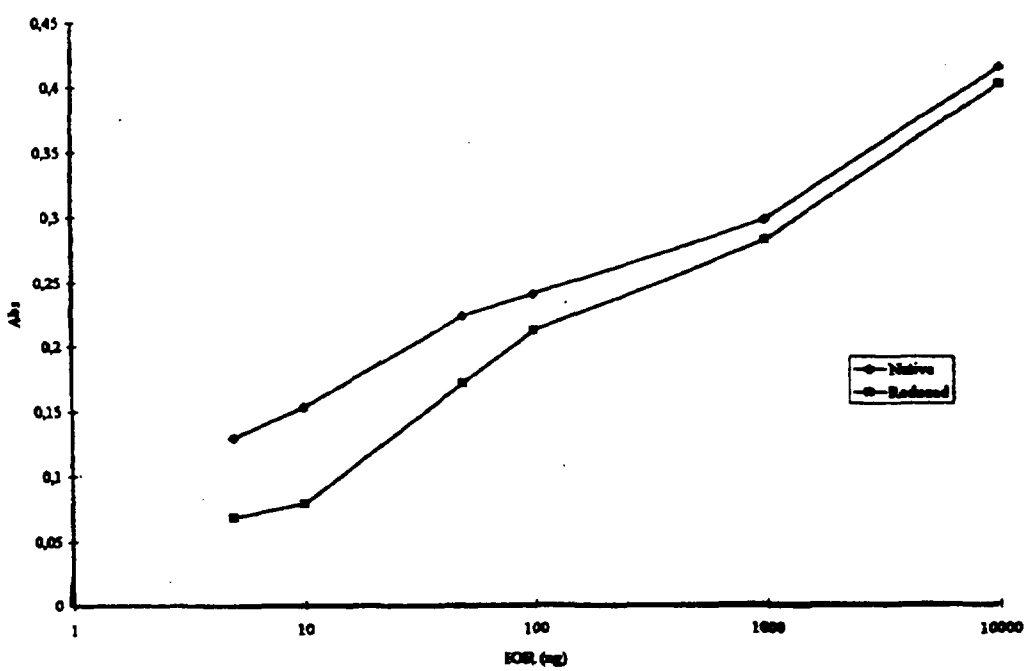


*FIG. 2. ELISA comparing the binding of the reduced and native antibody with the purified antigen.*

**2.1.3. Results**

*2.1.3.1. Direct labelling and quality control of ior-CEA-1*

Labelling efficiency values were higher than 95%. The HPLC radiochromatogram profile showed a single peak at 7.65 min (96%, recovery 100%) without fragments, aggregates or colloids. SDS PAGE under nonreducing conditions showed a fragmentation of the protein. 2-ME reduction followed by denaturing SDS PAGE produced fragments and comparison with the markers indicated one peak at 116.5 kd and the other one at 49.5 kd and the absence of high molecular weight species. The label is clearly not on IgG but is distributed among three lower molecular weight species and autoradiographies showed that the majority of the radioactivity was associated with the 116.5 kd peak. The instability to cysteine challenge is dependent on the temperature and this antibody showed a similar behavior towards cysteine relative to other ME reduced antibodies. Perhaps it would be necessary to perform this challenge at lower cysteine concentrations, to which the antibody may be expected to be exposed to, in vivo. Biodistribution studies showed the highest value for kidney levels (3.29±0.44). Levels in blood and in other tissues tended to be lower probably in concordance with the instability showed to the cysteine challenge. Figure 1 shows the results of the competitive binding assay. Comparison of the curve shapes showed that the reduced antibody competes with binding as efficiently as the non reduced antibody. Figure 2 shows a similar curve for the reduced and native antibody with the ELISA assay.

*2.1.3.2. Labelling and quality control of lyophilized IgG kit*

Values for labelling efficiency of labelled IgG were higher than 95% SDS PAGE was performed in all cases and showed fragmentation of the protein. There was a slight difference between the two molar ratios. When the 500:1 ratio was used the radioactivity associated with the 116.5 kd peak was even higher. Lyophilized preparations were labelled and the labelling efficiency was higher than 95% (2% of aggregates). Biodistribution results in normal mice and in the animal model are listed in Table I and Table II respectively.

TABLE I. BIODISTRIBUTION (% ID/gm) IN NORMAL BALB/c MICE AT 4 AND 24 h POST INJECTION OF $^{99}Tc^{m}$ LABELLED IgG (KIT)

| ORGAN | % I.D./gm at 4 h | % I.D./gm at 24 h |
|---|---|---|
| Blood | 28.1±3.0 | 14.5±2.6 |
| Liver | 11.7±1.8 | 6.3±1.9 |
| Spleen | 7.7±1.6 | 4.4±1.6 |
| Kidneys | 22.4±2.8 | 13.2±0.9 |
| Intestine | 4.1±0.9 | 1.2±0.3 |
| Lung | 8.4±1.8 | 5.3±0.9 |
| N.T. | 4.5±0.8 | 2.3±0.5 |

TABLE II. BIODISTRIBUTION (% ID/gm) IN INDUCED INFLAMMATION MICE AT 4 AND 24 h POST INJECTION OF $^{99}Tc^{m}$ LABELLED IgG (KIT)

| ORGAN | % I.D./gm at 4 h | % I.D./gm at 24 h |
|---|---|---|
| Blood | 30.5±2.5 | 16.3±1.8 |
| Liver | 14.8±1.6 | 8.2±1.2 |
| Spleen | 10.3±1.2 | 6.1±0.9 |
| Kidneys | 27.3±1.8 | 5.2±1.3 |
| Intestine | 8.3±0.9 | 2.5±0.5 |
| Lung | 9.5±1.4 | 5.8±1.8 |
| I.T. | 10.8±1.3 | 4.9±0.8 |
| N.T. | 4.6±0.5 | 2.6±0.3 |

Biodistribution results showed higher kidney levels than liver levels and the ratio of I.T./N.T. was 2.3 at 4 h and 1.9 at 24 h.

Stability towards storage gave labelling efficiencies higher than 95% in all cases and no differences were observed between the two SDS PAGE autoradiographies.

*2.1.3.3. Labelling and quality control of lyophilized ior-CEA-1*

Lyophilized preparations of ior-CEA-1 were labelled with the required activity of $^{99}Tc^{m}$ after reconstitution of the kit with 2 mL of saline. Values for labelling efficiency were higher than 95%. Figure 3 shows a competitive binding assay comparing the lyophilized antibody with the native one.

*2.1.3.4. Labelling and quality control of photoactivated IgG*

Figure 4 shows the effect of the amount of tin per 500 μg of protein on the labelling efficiency. Higher amounts than 23 μg of tin gave between 87 and 93% labelling. Controls where IgG and MDP/tin were not irradiated but labelled after 20 min. and quality control tested after 30 min. gave between 7 and 10% labelling. Other controls were IgG was irradiated alone and MDP/tin was added to the antibody after irradiation gave very low labelling yields but better labelling was obtained when MDP was added after the irradiation of IgG and tin. It seems that tin is required during the UV irradiation. A typical photoactivation experiment was performed in two vials but the labelling was delayed 30 min in one case and 2 h in the other one. Labelling yield higher than 95% was obtained when the photoactivated IgG was labelled after 2 h. It seems that a pretinning approach was achieved but with a low amount of tin.

Figure 5 shows the effect of the IgG concentration on the labelling efficiency. Concentrations between 1.5 and 5 mg/mL gave 90% labelling yields but more reproducible results were obtained at concentrations higher than 5 mg/mL.

Labelling efficiencies higher than 95% were obtained for bulk irradiation and were more reproducible than single vial irradiation. Aliquots from bulk irradiation stored at -20°C could be labelled at high labelling efficiencies (>95%) for upto 10 days and for upto 1 month when the vials were stored at -70°C.

Figure 6 shows the cysteine challenge of two labelled IgG. Stability of the $^{99}Tc^{m}$ labelled photoactivated IgG was similar to that of the $^{99}Tc^{m}$ labelled mercaptoethanol reduced IgG.

Biodistribution results in normal Balb/c mice are listed in Table III.

Biodistribution results in mice with a sterile turpentine induced inflammation are listed in Table IV.

TABLE III. BIODISTRIBUTION (% ID/gm) IN NORMAL BALB/c MICE AT 4 AND 24 h POST INJECTION OF $^{99}Tc^{m}$ LABELLED PHOTOACTIVATED IgG

| ORGAN | % ID/gm at 4 h | % ID/gm at 24 h |
|---|---|---|
| Blood | 18.5±2.2 | 8.1±1.1 |
| Liver | 10.8±0.9 | 4.5±0.3 |
| Spleen | 4.8±1.6 | 1.1±0.1 |
| Kidneys | 25.0±2.7 | 8.7±1.3 |
| Intestine | 5.5±0.7 | 1.6±0.5 |
| Lung | 6.6±0.8 | 1.0±0.3 |
| N.T. | 3.3±1.1 | 1.9±0.3 |

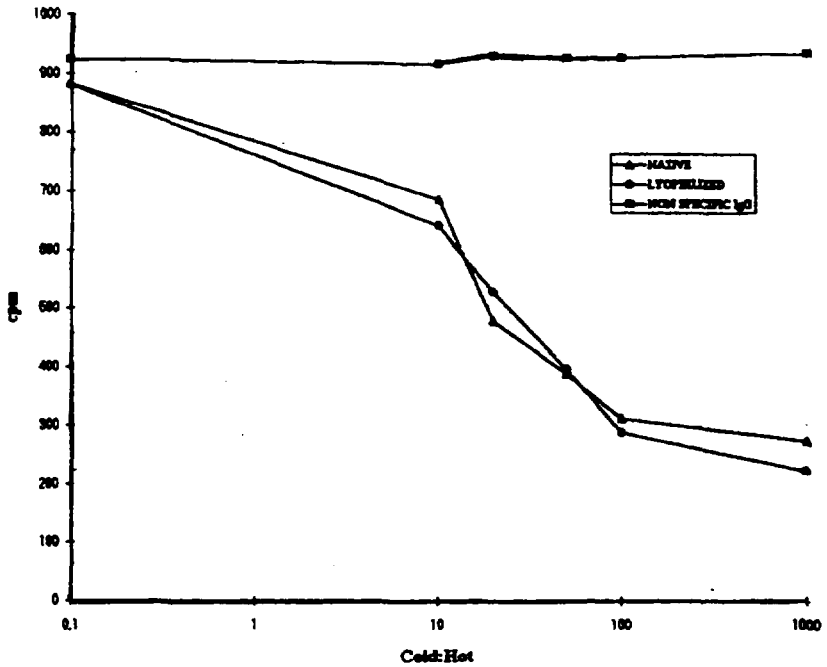


*FIG. 3. Competitive binding assay comparing the lyophilized reduced antibody with the native and non-specific IgG.*

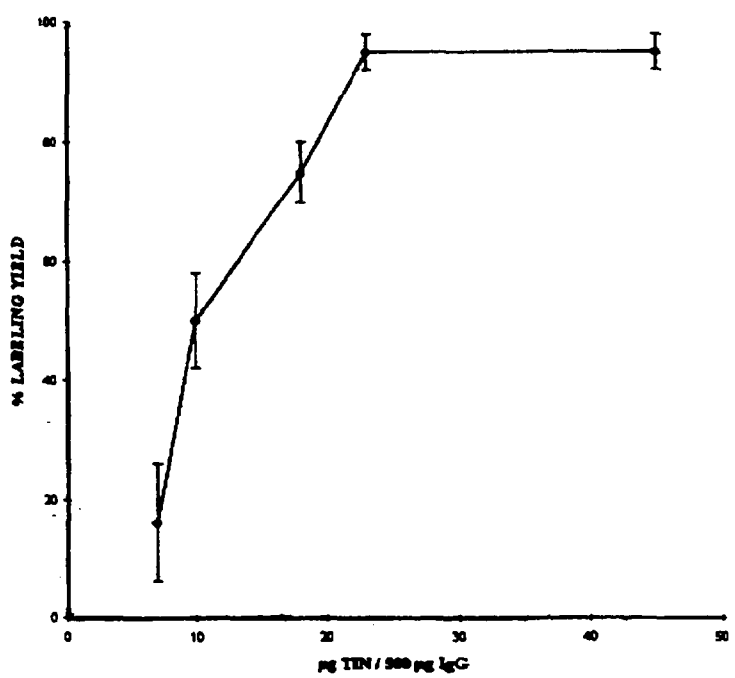


*FIG. 4. The effect of the tin-antibody ratio on labelling efficiency.*

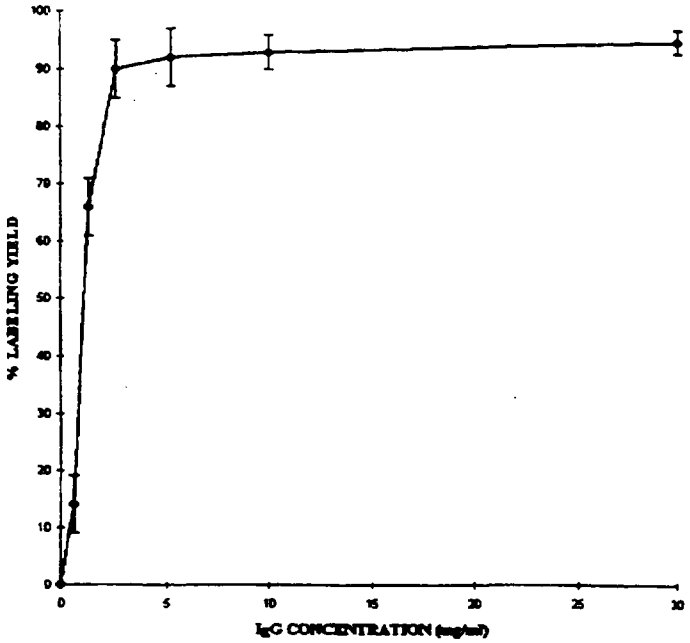


*FIG. 5. The effect of antibody concentration on labelling efficiency.*

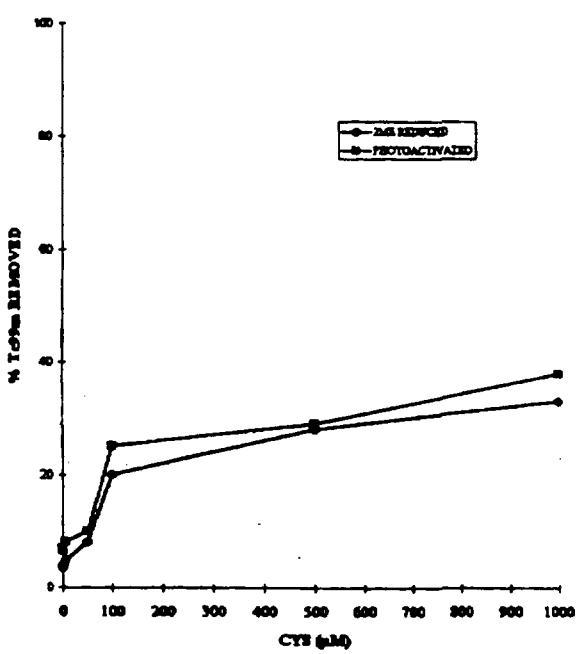


*FIG. 6. Percent of $^{99}Tc^{m}$ removed from IgG versus cysteine concentration at 37°C for photoactivated IgG and 2-ME reduced IgG.*

TABLE IV. BIODISTRIBUTION (% ID/gm) IN INDUCED INFLAMMATION MICE AT 4 AND 24 h POST ADMINISTRATION OF $^{99}Tc^{m}$ LABELLED PHOTOACTIVATED IgG

| ORGAN | % ID/gm at 4 h | % ID/gm at 24 h |
|---|---|---|
| Blood | 20.7±2.6 | 9.4±1.5 |
| Liver | 9.5±1.2 | 4.6±0.7 |
| Spleen | 4.9±1.2 | 3.5±0.3 |
| Kidneys | 26.2±3.7 | 11.5±1.8 |
| Intestine | 5.1±1.3 | 1.1±0.1 |
| Lung | 9.3±2.4 | 3.9±1.2 |
| N.T. | 2.4±0.5 | 1.4±0.2 |
| I.T. | 5.5±0.8 | 2.5±0.4 |

Kidney levels were higher than liver levels in both animal models and there was no significant difference in uptake of most tissues between the two groups . Results were similar to that obtained from distribution of $^{99}Tc^{m}$ labelled mercaptoethanol reduced IgG (control). However, the label cleared slightly faster from blood and some tissues than the control. The clearance in blood between the two points was 2.4 and 1.9 for labelled photoactivated IgG and the control respectively. The percentage for whole body retention during 5 h was 70±5.0 for labelled photoactivated IgG and 76±6.7 for the control. This difference is also an evidence of the more rapid clearance for the photoactivated IgG. Because of the short physical half-life of $^{99}Tc^{m}$, this clearance may be an advantage if there is no reduction in the uptake in tumour. In this case the label cleared from the inflammation site with a similar rate of 2.2 for labelled photoactivated IgG and even greater (2.2) than blood clearance (1.9) for the control. The ratio between IT/NT was similar and independent of the method of labelling.

*2.1.3.5. Indirect labelling of IgG*

Experiments were carried out to conjugate the protein at different molar ratios 0:1, 10:1 and 50:1 (NHS-$MAG_3$:IgG) and at different pH, in order to obtain the proper conditions for conjugation. Labelling of these complexes were achieved before and after removing the uncomplexed chelator. The control for non-specific labelling was the 0:1 molar ratio. In addition identical labelling experiments were performed to assess the binding of $^{99}Tc^{m}$ to glucoheptonate and to NHS-$MAG_3$ by ligand exchange from the labelled GH. Radiochemical purity was evaluated by HPLC and ITLC.

Labelling yields for the different controls were 98% for glucoheptonate, 95–98% for the NHS-$MAG_3$ labelled by ligand exchange from glucoheptonate and 2–3% for native IgG (non-specific labelling). Conjugation of IgG with the NHS $MAG_3$ chelator and labelling after purification was better achieved at a molar ratio of 50:1. Typically 1–2 mg of protein was conjugated and different amounts of conjugated protein (100–500 ug) with a concentration of approximately 0.5 mg/mL were used for labelling procedures. Labelling efficiency was usually 70–80% and purification of the labelled product was carried out using a centrifugal filter. Radiochemical purity as determined by HPLC and ITLC was 95–98% with 100% recovery. The radiochromatographic profile after labelling showed one prominent peak of labelled IgG, small amounts of radiolabelled aggregates of high molecular weight (3%) and radiolabelled species of low molecular weight (20%). This last percentage was in agreement with that obtained by ITLC in saline. As $^{99}Tc^{m}$-glucoheptonate is retained by the HPLC column and recovery was nearly 100%, radiolabelled species of low molecular weight would be rather different than labelled glucoheptonate and they were equally removed after purification.

Transchelation to cysteine was studied at five molar ratios, cysteine: IgG ranging from 908:1 to 0.45:1 and the percentage of dissociation ranged from 6.2 to 1.2% respectively. The percentage of $^{99}Tc^{m}$ removed from labelled IgG via 2-ME reduction varied from 35 to 3.6% for the lower molar ratio and from 38 to 6.5% in the case of $^{99}Tc^{m}$ labelled photoactivated IgG. These two direct labels were more susceptible to transchelation than the indirect label. Results are shown in Fig. 7.

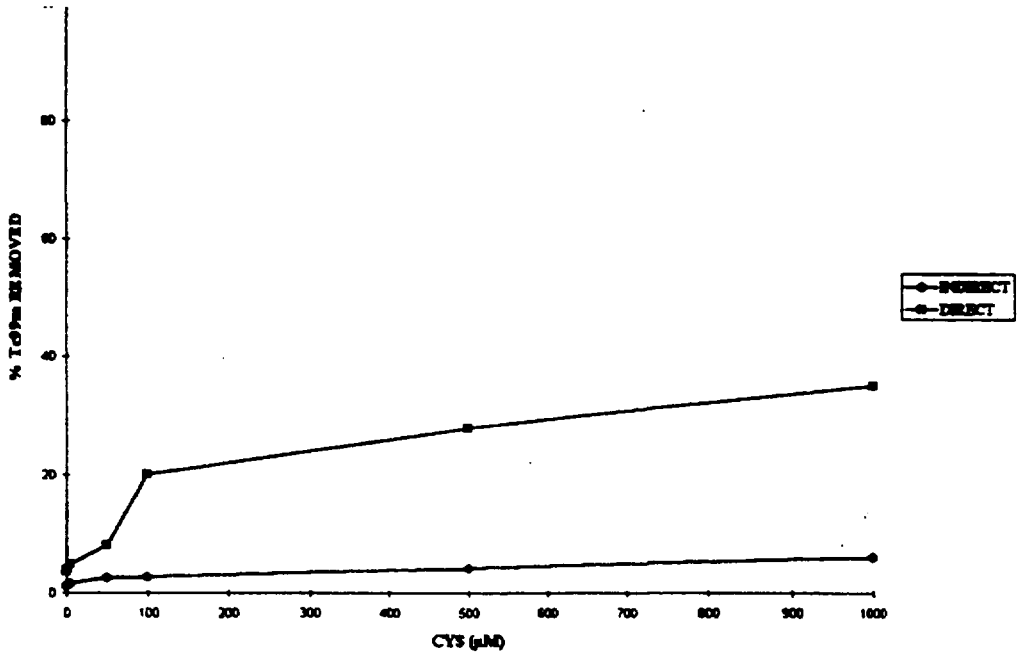


*FIG. 7. Percent of $^{99}Tc^{m}$ removed from IgG versus cysteine concentration at 37°C for directly and indirectly labelled IgG.*

Biodistribution results in normal mice at 4 and 24 h are listed in Table V. No significant differences were observed in uptake of the proteins by any organ in both animal models. Liver levels were slightly higher than kidney levels. Comparison in biodistribution at the same points with IgG labelled via 2-ME reduction were carried out in both animal models. Differences with labelling methods were evident, liver levels were lower and kidney levels were higher for the 2-ME relative to the NHS $MAG_3$ labelled IgG. The label cleared more rapidly from blood and tissues after direct labelling. The whole body activity levels measured in normal Balb/c mice at 1 h intervals were different. The percentage of the injected activity remaining in the animal at 4 h was 76±6.7 for directly and 82.4±2.6 for indirectly labelled IgG. This simplified conjugation procedure for acetyl-protected.

NHS-$MAG_3$ may provide an alternative (if chelator is available) for radiolabelling antibodies and other amines with $^{99}Tc^{m}$.

TABLE V. BIODISTRIBUTION (% ID/gm) IN NORMAL BALB/c MICE AT 4 AND 24 h POST ADMINISTRATION OF INDIRECTLY LABELLED $^{99}Tc^{m}$-IgG

| ORGAN | % ID/gm at 4 h | % ID/gm at 24 h |
|---|---|---|
| Blood | 22.3±1.3 | 15.9±2.6 |
| Liver | 9.5±2.3 | 7.4±1.1 |
| Spleen | 7.2±1.5 | 5.1±0.2 |
| Kidneys | 8.4±1.5 | 7.7±1.6 |
| Intestine | 4.8±0.1 | 2.4±0.5 |
| Lung | 9.5±3.4 | 7.5±1.3 |
| N.T. | 3.1±1.0 | 2.6±0.8 |

TABLE VI. BIODISTRIBUTION (% ID/gm) IN INDUCED INFLAMMATION MICE AT 4 AND 24 h POST ADMINISTRATION OF INDIRECTLY LABELLED $^{99}Tc^{m}$-IgG

| ORGAN | % ID/gm at 4 h | % ID/gm at 24 h |
|---|---|---|
| Blood | 25.2±2.9 | 17.8±0.74 |
| Liver | 8.7±0.8 | 8.2±1.1 |
| Spleen | 7.8±1.7 | 3.6±0.9 |
| Kidneys | 8.2±2.2 | 6.1±0.1 |
| Intestine | 4.9±0.5 | 1.4±0.1 |
| Lung | 9.0±1.1 | 5.6±0.2 |
| N.T. | 2.9±0.1 | 2.0±0.7 |
| I.T. | 5.8±0.3 | 3.7±0.4 |

### 2.1.4. Conclusions

Two direct labelling methods and one indirect method for radiolabelling monoclonal and polyclonal antibodies with $^{99}Tc^{m}$ were used. The principal advantage of the indirect method is that it can be employed with a wide range of biomolecules in contrast to direct methods which can be used only for labelling large molecules. Many chelating agents are generally not available and purification steps are required for indirect methods. The main advantage of the direct approach is its simplicity and the possibility of developing kit formulations that can be labelled in any Nuclear Medicine Center for diagnostic studies.

From the results described above it is clear that the ior- CEA-1 antibody can be directly labelled with $^{99}Tc^{m}$ using 2-ME as the reducing agent with a high radiochemical purity and without damage to the antigen-binding ability of the antibody. It is also clear that a lyophilized, sterile, pyrogen-free preparation under nitrogen atmosphere and ready for one step labelling of ior-CEA-1 and IgG with $^{99}Tc^{m}$ was achieved.

Further studies are required to establish the properties of photoactivated as well as the indirect labelling techniques for tumour imaging.

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