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LUCAS HEIGHTS RESEARCH LABORATORIES

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ABSTRACT

The development of a dysprosium-165 product, Dy-HMA, which is suitable for the radiation synovectomy of arthritic joints is described. Dysprosium-165 is a short-lived ($t_{1/2}$ = 139 min) beta-emitter produced by the neutron irradiation of natural dysprosium. Dy-HMA is a suspension of macroaggregated hydroxide particles in saline with the majority of particles in the 3-5 μm range.

Studies in rabbits have demonstrated minimal leakage following the intra-articular injection of a knee joint. At 24 hours, the accumulation in the liver is about 0.003% of the injected dose and there is considerably less in other organs and tissue.

The use of Dy-HMA has considerable advantages over the presently used yttrium-90 products. The undesired leakage to and subsequent irradiation of other organs is considerably reduced. The period of hospitalisation is reduced from four days to one and the production of ^{165}Dy in Australia will overcome the difficulties of supply of ^{90}Y from overseas.

EDITORIAL NOTE

The Australian Nuclear Science and Technology Organisation replaced the Australian Atomic Energy Commission on 27 April 1987. Reports issued after April 1987 have the prefix ANSTO with no change of the symbol (E, M, S or C) or numbering sequence.

CONTENTS

1. INTRODUCTION

Rheumatoid arthritis of the knee causes significant pain and usually disability because more than half of the patients develop chronic inflammation of the synovial membrane of the knee joint [Fleming *et al.* 1976]. Aspirin, non-steroidal anti-inflammatory agents and intra-articular injection of corticosteroids are initially employed to control the inflammation, but in cases where this treatment is unsuccessful, the inflamed synovial membrane must be removed.

Surgical synovectomy provides relief for 2-5 years [Laurin *et al.* 1974] but this treatment has many disadvantages. Apart from the risks associated with surgery, there is also a long period of hospitalisation and rehabilitation, especially for older patients. During the last 15 years, radiation synovectomy has increasingly been used as an alternative to surgery. This procedure involves the injection of a β-emitting radionuclide, in some particulate form, into the synovial sac of the knee joint. Radionuclides that emit beta particles are preferred because the volume of energy deposition is small with a maximum depth penetration of 1 cm or less.

Although many β-emitting radionuclides have been investigated [Sledge *et al.* 1984], yttrium-90 (^{90}Y) and gold-198 (^{198}Au) colloids have become the most frequently used. They are extensively used in Europe, and in Australia it is estimated that 10-15 patients are treated each week with a 185 MBq injection of yttrium-90 silicate colloid, which is sold by Amersham Australia Pty Ltd.

Although radiation synovectomy is effective in the treatment of chronic rheumatoid synovitis of the knee [Virkkunen *et al.* 1967; Spooren *et al.* 1985] it has not obtained widespread acceptance because of leakages of radioactivity from the joint which results in exposure to normal tissues. When coupled with the relatively long half-lives of ^{90}Y and ^{198}Au (approximately 2.7 days), this leakage can produce significant integrated doses to the liver, kidney and especially the regional lymph nodes. With a 10% leakage to a 10 g lymph node [Gumpel *et al.* 1975], the injection of 185 MBq of ^{90}Y silicate or 370 MBq of ^{198}Au colloid will result in integrated doses of 9100 and 6380 cGy, respectively.

Over the past 10 years, studies in the USA [Hnatowich *et al.* 1978; Sledge *et al.* 1977, 1984; Zalutsky *et al.* 1986] have shown that leakages from the joint and the subsequent undesired radiation doses to other organs can be significantly reduced by the use of the shorter half-life radionuclide, ^{165}Dy ($t_{½}$ = 139 min). The formulation dysprosium-ferric hydroxide macroaggregates (Dy-FHMA) has the majority of its particles in the range 1-5 μm in diameter, which is approximately 100 times larger than the ^{90}Y or ^{198}Au colloids. It appears that the greatly reduced leakage of Dy-FHMA can be attributed to this larger particle size [Sledge *et al.* 1986]. When combined with the shorter half-life of ^{165}Dy, these reduced leakages significantly lower the radiation doses to other organs.

In **table 1**, a comparison is given for the radiation doses due to leakage of Dy-FHMA and Y-silicate colloid. Although the doses from Dy-FHMA are less than half for the whole body and liver, an even more significant reduction in dose is obtained for the regional lymph nodes. Apart from the reduced radiation doses to other organs, the use of a ^{165}Dy product offers the additional advantage that patients can be discharged from hospital in a day or less [Sledge *et al.* 1984] whereas patients treated with the longer-lived ^{90}Y must be hospitalised for 3 to 7 days [Deckart *et al.* 1979]. In addition, the production of ^{165}Dy in Australia would avoid the difficulties of supply that have been experienced for ^{90}Y silicate which is flown in from England. This has been a particular problem for hospitals in Perth.

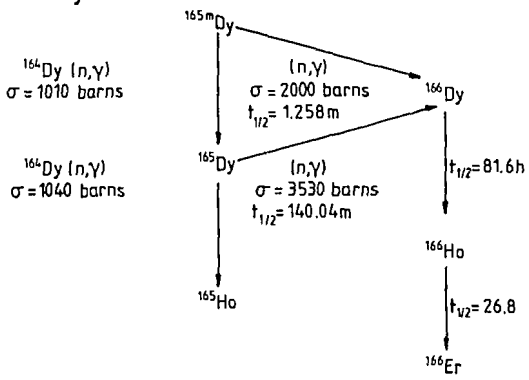
The benefit obtained from the use of Dy-FHMA in other countries has prompted ANSTO to undertake the development and marketing of a ^{165}Dy product for use in Australia.

2. TARGET ACTIVATION AND RADIONUCLIDIC PURITY

Dysprosium-165 is produced by the thermal neutron activation of ^{164}Dy. Spectrographically standardised Dy_2O_3 is used as the target material. Hnatowich *et al.* [1978] used a thermal neutron flux of 4.0 x 10^{13} n cm^{-2} s^{-1} and an irradiation time of eight hours. The ^{165}Dy-FHMA was prepared for injection at two hours post irradiation. Available 'self service' fluxes in the ANSTO 10 MW research reactor HIFAR are 5.0 - 7.0 x 10^{12} and 5.0 x 10^{13} n cm^{-2} s^{-1}. Activation was investigated at both fluxes.

The neutron activation of ^{164}Dy is shown in **figure 1**. The cross sections are from Sekine and Baba [1981] and Mughabghab [1984]. Dysprosium-165 has a large activation cross section; ^{166}Dy ($t_{½}$ = 81.6 h) and its daughter ^{166}Ho ($t_{1/2}$ = 26.8 h) are also formed during irradiation. A computer program was used to

calculate the activation of ^{164}Dy. The formation of ^{166}Dy-^{166}Ho was calculated using the equations for double neutron capture and the equations for the growth and decay of the radioactive daughter. The calculated activities relative to a therapy dose of 11 GBq of ^{165}Dy for times up to eight hours after an eight-hour irradiation in a flux of 5.0 x 10^{12} n cm^{-2} s^{-1} are given in **table 2**. The table also shows the ^{166}Dy-^{166}Ho activities resulting from an experimental irradiation of a 5 mg Dy_2O_3 target. These activities are also expressed relative to 11 GBq of ^{165}Dy.



The ^{165}Dy activity was determined by ionisation chamber measurement (see **section 5.1**). The ^{166}Dy-^{166}Ho activities were determined by gamma spectrometry using a calibrated system based on an intrinsic germanium detector. In a flux of 5.0 x 10^{13} n cm^{-2} s^{-1}, the ^{166}Dy-^{166}Ho activities will be 10 times those given in **table 2** since activation by secondary neutron capture is proportional to the square of the flux.

In addition to ^{166}Dy and ^{166}Ho, a number of radionuclidic impurities resulting from the activation of chemical impurities in the target were detected. These were identified and their activities measured by gamma spectrometry after decay of ^{165}Dy. The measurement sample, contained in a standard 10 mL counting vial, was counted periodically over approximately two months to permit impurity identification by half-life as well as by gamma peak energy. The impurity activities present at two hours and eight hours post irradiation are given in **table 3**. The percentage of impurities other than ^{166}Dy and ^{166}Ho will be independent of flux. Hnatowich *et al.* [1978] claimed that the only impurity detected was ^{24}Na, whereas in their paper on the radiation dosimetry of ^{165}Dy-FHMA, Zalutsky *et al.* [1986] quoted the radionuclidic purity as 99.99 per cent but did not mention specific impurities. Our measurements show that the radionuclidic purity for Dy_2O_3 irradiated for eight hours in a flux of 5.0 x 10^{12} n $cm^{-2}s^{-1}$ was better than 99.99 per cent for decay up to approximately 8 hours post irradiation.

3. CHOICE OF IRRADIATION CONDITIONS

3.1 Specific Activity

As applied by Hnatowich *et al.* [1978], the use of ^{165}Dy-FHMA involves the injection of approximately 2.5 mg of dysprosium. This can be achieved by an eight hour irradiation and a flux of 5 x 10^{12} n cm^{-2} s^{-1} provided that the medical centre is close to the production site. In the proposed use of ANSTO produced ^{165}Dy at hospitals, the combined processing and transport times would be up to ten hours post irradiation. The long decay times will cause a reduction in ^{165}Dy specific activity and, for the required 11 GBq therapy dose, an increase in the mass of dysprosium to be injected.

Since the thermal neutron flux is not known precisely and since flux depression can lower the ^{165}Dy produced, the ^{165}Dy specific activity, and hence the mass of dysprosium required for the injection of 11 GBq ^{165}Dy, was determined experimentally. The normal irradiation conditions used were

Thermal neutron flux	5.0 x 10^{12} n cm^{-2} s^{-1}
Irradiation time	8.0 h
Target mass	5 mg Dy_2O_3

The specific activity produced was 7.66 GBq mg^{-1} of dysprosium and the mass of dysprosium required for the injection of 11 GBq ^{165}Dy at post irradiation times up to 10 hours is given in **table 4**. To maintain the specific activity close to that used by Hnatowich *et al.* for all proposed decay times, it would be necessary to irradiate the target in a flux of 5.0 x 10^{13} n cm^{-2} s^{-1}. The corresponding dysprosium masses for a typical

therapy dose are also given in **table 4.**

3.2 Radiation Dose Due To Impurities

Of the radionuclidic impurities present in irradiated ^{165}Dy only ^{166}Dy and ^{166}Ho result in a significant radiation dose both to the knee and to other organs owing to leakage from the injection site. Zalutsky *et al.* [1986] have calculated the dose to the knee and several other organs from the injection of ^{165}Dy as ferric hydroxide macroaggregates. Since the hydroxide compound shows similar or lower leakage rates, it was decided that the model described by *Zalutsky et al.* [1986] could be used to estimate the doses due to ^{166}Dy and ^{166}Ho impurities.

For the calculated impurity activities given in **table 2**, the radiation dose to the knee, whole body, liver, lymph nodes and ovaries were found for ^{165}Dy and ^{166}Ho produced by an eight-hour irradiation in a flux of 5.0 x 10^{12} n cm^{-2} s^{-1}. It was assumed that the leakage rates for ^{166}Dy and ^{166}Ho were the same as those for ^{165}Dy. The ^{165}Dy and impurity doses corresponding to 11 GBq ^{165}Dy injected at two hours and eight hours post irradiation are given in **tables 5** and **6.**

For irradiation in a flux of 5.0 x 10^{13} n cm^{-2} s^{-1}, the impurity doses would be increased by a factor of 10 on the values given in the tables for a flux of 5.0 x 10^{12} n cm^{-2} s^{-1}. Even for the high flux irradiation, the combined ^{165}Dy and impurity leakage doses are considerably less than those experienced with ^{90}Y (**table 1**) and other nuclides used for radiation synovectomy. It was decided however to restrict the dose to the lowest level possible by irradiation. Even though this choice requires the injection of up to 30 mg of dysprosium this should not be a problem because of the low toxicity of this element (see **section 4**).

4. CRITERIA FOR THE CHEMICAL FORMULATION OF A ^{165}Dy PRODUCT

For the preparation of Dy-FHMA, 2 mg of dysprosium nitrate, which had been irradiated in a neutron flux of 4 x 10^{12} n cm^{-2} s^{-1} for six hours, is co-precipitated with 1 mg of ferrous sulphate. In this case the ferric hydroxide product acts as a carrier for the dysprosium. The patient dose in the range 9-11 GBq is calculated as being equivalent to 185 MBq ^{90}Y when corrections are made for the differences in half-life and beta energy [Sledge *et al.* 1984].

For the US studies, the nuclear reactor at the Massachusetts Institute of Technology is located very close to the hospital department in which the studies are conducted. The production and distribution of a ^{165}Dy product for city centres in Australia, however, requires a rather different approach to the formulation of the product. In **table 7** are shown the amounts of dysprosium required to obtain an injected dose of 11 GBq for patients located in the major Australian cities. A correction is made for the decay time of ^{165}Dy after removal from HIFAR until the time the patient receives the injection. As described more fully in **section 3.1**, for these calculations, 7.66 GBq mg^{-1} was taken as the most suitable specific activity of ^{165}Dy. The decay time is comprised of the flight time, plus

Chemical processing time	1.5 h
Packaging and transport to Mascot Airport	1.0 h
Either airport handling for interstate hospitals or transport to Sydney hospitals	2.5 h

From **table 7**, it can be seen that the amount of dysprosium needed for a 11 GBq dose ranges from 6 mg for a patient in Sydney to 30.5 mg for a patient in Perth. For these quantities of dysprosium, a chemical carrier is not required since 6 mg of dysprosium hydroxide can be readily handled. Thus the development of a ^{165}Dy product for use in Australia is based solely on the preparation of dysprosium hydroxide macroaggregates (Dy-HMA) suspended in saline. Fortunately the toxicity of both Dy and Ho is very low [Haley 1965; Bruce *et al.* 1963]. The LD_{50} (mg/kg) for dysprosium chloride and holmium chloride for mice given an intraperitoneal injection is 310 and 320, and for rats 295 and 270, respectively. An injection containing 30.5 mg of Dy for a 65 kg patient would be in the range 0.2 - 0.3% of the expected LD_{50} dose.

Compared with the preparation of Dy-FHMA [Sledge *et al.* 1986], the elimination of ferric hydroxide in the preparation of Dy-HMA not only makes the preparation procedure simpler and eliminates any quality control on the ferrous material, but there are also medical advantages. There is evidence to suggest that the presence of iron in the synovial sac can cause further inflammation of the synovial membrane [Blake *et al.* 1981].

From the studies with Dy-FHMA [Sledge *et al.* 1984; Hnatowich *et al.* 1978], it is apparent that particle size has a significant effect on minimising leakage. With Dy-FHMA, the majority of particles are in the 0.5 - 5 μm range. Although it appears most important to have a negligible number of particles less than 0.5 μm, in order to minimise leakage, particles of approximately 5 μm may not be of great significance. Nevertheless, for Dy-HMA, the method of preparation was developed so as to produce particles in the same range as Dy-FHMA. At least this gives a more useful comparison of the leakages observed with similar animal studies.

5. METHODS

5.1 Activity Measurements

To ensure the accurate administration of the radiation dose to the synovium, it is necessary to establish a calibration for the measurement of ^{165}Dy activity. At the present time an absolute standard of ^{165}Dy is not available and, because of the short half-life, direct inter-comparisons with other users are not possible. The activity calibration was established using accepted 'working standards' procedures.

Although primarily regarded as a beta-emitter, the decay scheme of ^{165}Dy includes 54 low abundance gamma rays [Sowby 1983]. These, together with the bremsstrahlung contribution from the beta emissions, provide sufficient output for the measurement of therapy doses of ^{165}Dy in a calibrated ionisation chamber of the type used by ANSTO for the routine measurement of medical radioisotopes. A working ionisation chamber calibration factor was calculated from the decay scheme and the known ionisation chamber gamma energy response using the method described by Bellinger and Hetherington [1976]. The calibration factor included a bremsstrahlung contribution based on the response of the chamber to pure beta-emitters under the standard geometry (10 mL solution in counting vial) used for measurement.

This calibration was confirmed by measurements made by ANSTO's Radioisotope Standards Group. The ionisation current for a ^{165}Dy solution was measured in the chamber used for routine activity measurement. The activity was then determined using the working standard procedure described by Urquhart [1986]. After correcting the measurements to a common reference time, the ionisation chamber calibration factor was calculated. For a nuclide such as ^{165}Dy, which has a complex decay scheme, the overall uncertainty in the calibration factor is given by Urquhart as ± 5 per cent. The uncertainty is further increased by the need to include the significant bremsstrahlung contribution (approximately 10 per cent of the ionisation current). Therefore the overall uncertainty in the calibration is of the order of ± 10 per cent. Despite the large uncertainty, the calculations found by the two methods agreed to within 1 per cent.

Gamma spectrometry was also used to determine ^{165}Dy activity for comparison with that found by the ionisation chamber methods. Using a germanium detector for which the energy response and efficiency for energies up to ~ 2 MeV had been determined with gamma ray standards, the activity of a ^{165}Dy solution was determined from the 0.2798, 0.3617, 0.6334 and 1.080 MeV photopeaks. The results are given in **appendix A**. The activities given for each energy represent the mean of the values found in nine separate counts of the sample taken over a period of eight hours. All activities have been decay-corrected to a common reference time. Again the overall uncertainty is of the order of ± 10 per cent and the measured activity agrees within the uncertainty limits with the ionisation chamber measurement.

5.2 Chemical Formulation Analysis

Natural dysprosium was used for all studies and, to facilitate counting, was spiked with tracer amounts of the chemically identical radioisotopes gadolinium-153 ($t_{½}$ = 242 d) or samarium-153 ($t_{½}$ = 46.8 h). The ^{153}Gd spike was used for the chemical formulation development and the stock solution was prepared by dissolving neutron irradiated Gd_2O_3 in HCl and adjusting the pH to 3.0 with NaOH. The working solution had a concentration of approximately 1.7 μg mL^{-1}, and typically 0.14 mL was added to the dysprosium before precipitation, to give between 150 000 - 200 000 counts in 6 s for 1 mL of the final preparation.

To avoid lengthy storage of carcasses until the residual activity reduced to background, ^{153}Sm was used as the tracer for the animal studies. Approximately 50 μg of Sm_2O_3 in a silica ampoule was neutron-irradiated for five days and the oxide dissolved in 10 mL of 0.01 *N* HCl. Typically 1 mL of this solution was used so as to give a final activity of approximately 1 MBq/0.1 mL. To compensate for the acid added with the ^{153}Sm spike, the volume of 0.5 *N* NaOH used for the precipitation was increased by the required amount.

Particle sizes were determined by counting the activity that would pass through 13 mm diameter Nuclepore polycarbonate membrane filters in the range 1-12 μm. Since ^{153}Gd, ^{153}Sm and ^{165}Dy have

essentially identical chemistry, it is reasonable to assume that the percentage of radioactivity that will pass through a membrane filter is an accurate measure of particle size. Only polycarbonate membrane filters were used in this range since it has been shown that polycarbonate filters give the most accurate estimate of particle sizes with the minimum of entrappment [Davis *et al.* 1974]. The more usual 0.45 μm Millipore cellulose filter was used to assess the amount of activity present as colloidal or free dysprosium.

Stock solutions of dysprosium were prepared by dissolving natural Dy_2O_3 in 0.01 *N* HCl and adjusting the pH to 3.0. Two stock solutions were prepared:

Solution A : 4.2 mg Dy mL^{-1}
Solution B : 10 mg Dy mL^{-1}

Counting of radioactivity was carried out on a 5 cm NaI well counter with a Canberra power supply, scaler and counter-timer. The suspensions were centrifuged in a Hettich 205 centrifuge.

6. DEVELOPMENT OF THE CHEMICAL FORMULATION

The initial development studies were carried out on a dose concentration of 0.5 mg Dy per mL of saline. This lower concentration avoided problems with the clogging of membrane filters that were used to size the particles. When the overall procedure had been developed, the method was confirmed using a patient dose of 6 mg Dy per mL of saline.

6.1 Basic Procedure - Formulation A

Dysprosium (0.5 mL of solution A), and gadolinium-153 (0.1 - 0.15 mL) were added to a 10 mL glass vial containing a magnetic stirrer bar. Five millilitres of saline was added and the mixture rapidly stirred.

Two millilitres of 0.1 *N* NaOH was added by syringe needle as quickly as possible. The suspension of dysprosium hydroxide was then transferred to a 13 mL plastic vacutainer tube and treated in an ultrasonic bath for 1 min. After centrifuging for 3 min at 240 g, the supernatant was decanted and the dysprosium hydroxide resuspended in 4 mL of saline. Samples were autoclaved at 132°C for 6 min then treated once more for 1 min in the ultrasonic bath.

With this method, precipitation occurred at approximately pH 12.0, the final pH was in the range 10.5 - 11.0 and the Dy concentration was 0.4 - 0.5 mg Dy mL^{-1}

6.2 Formulation B (Patient Dose)

Solution B (3 mL) was used instead of solution A and 1.2 mL of 0.5 *N* NaOH was used for the precipitation. The volume was made up to 10 mL, treated in an ultrasonic bath for 1 min and centrifuged at 240 g for 3 min.

The dysprosium hydroxide was twice more resuspended in 10 mL of saline and centrifuged for 1 min at a setting of 300 g. The particles were then suspended in 3 mL of saline, autoclaved at 132°C for 6 min and treated with ultrasonics for 1 min. The final pH was in the range 10.5 - 11.0, the volume was approximately 4 mL, and the Dy concentration was in the range 6 - 6.8 mg L^{-1}.

6.3 Results and Discussion

For formulations A and B, the percentage of ^{153}Gd activity incorporated in the suspension varied in the range 80 - 85, suggesting that this also is the efficiency of retention of dysprosium. Thus 10-15% of both ^{153}Gd and Dy are lost in the precipitation and decanting steps.

In **table 8** are shown the percentages of activity measured on various particle sizes for 10 batches of formulation A, four batches of formulation B, and two batches of formulation A for which ^{153}Sm was used as the tracer instead of ^{153}Gd. These results are compared with two literature studies of Dy-FHMA.

It can be seen that there is no significant difference in the particle size between formulations A and B. Furthermore, ^{153}Sm as tracer gives essentially the same results as for ^{153}Gd. The results suggest that formulation B may have slightly more particles greater than 12 μm but the reduced activity figures may also be due to greater clogging of the membrane filter. For both A and B, the majority of particles are in the 3-5 μm range with negligible activity being associated with particles less than 0.45 μm.

These results compare very well with the literature results for Dy-FHMA, especially since Dy-FHMA has a dysprosium-ferric concentration similar to formulation A. However, it is particularly important to note that Dy-HMA has significantly fewer particles less than 0.45 μm than Dy-FHMA. This would certainly assist the retention of Dy-HMA in a knee joint compared with Dy-FHMA.

The optimum ultrasonic bath treatment time of 1 min was chosen from the results shown in **table 9**. At least 30 s is required for more than 94% of particles to be less than 12 μm and after 1 min there is no significant change in size.

When the sample is autoclaved, there is a marked increase in particle size, but ultrasonic treatment for 1 min after autoclaving reduces the particle sizes to the acceptable levels (**table 10**). As can be seen from **table 8**, autoclaving at 121°C for 33 min also has no significant effect on particle size as long as the autoclaving is followed by ultrasonic treatment for 1 min.

When the samples of Dy-HMA are left to stand, there is no significant increase or decrease in particle sizes for 15 days (**table 11**). Formulation B (patient dose) was left to stand for 24 hours and again no significant change in particle size was observed (**table 12**).

The results of washout 'studies' are shown in **table 13**. Samples were rocked on a shaking machine, removed at various times, centrifuged at 400 g for 3 min and the supernatant passed through a 0.45 μm membrane filter and counted. At no time up to 24 h was the free activity greater than 0.02% and there was no marked change with time.

As shown in **table 14**, for method A, there is no appreciable effect on particle size from 0.2 - 9 mg of total Dy but after this, there is insufficient NaOH to precipitate enough of the Dy and the amount of activity less than 0.45 μm increases markedly.

No effect is observed if water is used instead of saline in the precipitation of dysprosium hydroxide and the temperature can be as high as 95°C. Dilution of the final sample by as much as a factor of 4 has no effect on particle size or free dysprosium (**table 15**).

6.4 Conclusions

Dy-HMA suspended in saline can be readily prepared with particle size ranges similar to those reported for Dy-FHMA. The method for preparation of Dy-HMA reported here, is much simpler than for Dy-FHMA. Dy-HMA can be autoclaved and is stable for at least twice the time it takes for the ^{165}Dy to decay to unusable levels.

7. BIOLOGICAL STUDIES IN RABBITS

Biological studies were carried out in rabbits on Dy-HMA spiked with trace levels of ^{153}Sm and ^{90}Y silicate from Amersham to determine the leakage of radioactivity to other organs following an intra-articular injection in the knee joint.

7.1 Method

Groups of three healthy male white rabbits (from Castle Hill Laboratory, Sydney University), each weighing about 3 kg, were used at each time interval and 2-3 time intervals were used in each experiment. Each rabbit was placed in a restraining box and anaesthetised with sodium pentabarbitone solution (30 mg/kg) injected intravenously *via* the lateral ear vein. When the animal was unconscious it was laid on its back and the hair in the region around each knee joint clipped.

In the case of the Dy-HMA, the product was found to settle very quickly, so the following injection technique was used. The stock of product was shaken well then approximately 0.2 mL loaded quickly into a 1.0 mL disposable plastic syringe and immediately counted in a Nuclear Associates Compucal dose calibrator. The syringe was then shaken well and approximately 0.1 mL injected into each knee joint, and the syringe recounted to estimate the injected dose.

For the ^{90}Y silicate, it was found that the doses could not be counted accurately enough using the dose calibrator owing to the physical characteristics of the emitted radiation, so the dose injected was estimated by weighing the syringe before and after injection. External standards were made by the same methods but the activity was injected into 50 mL plastic sample containers holding 20 mL of isotonic saline instead of knee joints.

The animals were replaced unrestrained in their cages, and allowed to regain consciousness. At 6, 24 and in some cases 72 hours post-injection the groups were sacrificed for tissue samples by an overdose of sodium pentabarbitone solution injected intravenously.

In the case of the animals treated with the Dy-HMA, samples of liver (20-30 g), kidneys (2), spleen, blood (20-30 mL), urine (residual in bladder), the three lymph nodes from each leg (ischiadial, inguinal, and popliteal grouped together), and the whole of each leg were taken, placed in 50 mL plastic sample tubes, weighed and counted in a large capacity sodium iodide well counter.

For the animals treated with ^{90}Y silicate, samples were taken as for the Dy-HMA but dried in a hot air oven at 65°C for one day, 95°C for one day, 105°C for one day then ashed at 250°C for two days, dissolved in 1 *N* HCl and diluted to 250 mL. Aliquots were then taken, mixed with Instagel, cooled and counted in a Packard liquid scintillation counter at suitable windows.

In both cases the results were calculated using external standards corrected for the small differences in injected dose, as indicated by the dose calibrator measurements or differences in syringe weights.

7.2 Results and Discussion

The biodistributions on each rabbit sacrificed at six hours after injection for three batches of formulation A and one batch of formulation B are given in **table 16**. The results at 24 hours for the same batches are given in **table 17**. These individual results have been included in this report to demonstrate the consistency of results not only between rabbits for each batch but between batches.

The average and range of these results are given in **table 18** and are compared with the results reported by Sledge *et al.* [1978] for Dy-FHMA. The leakages to the organs studied, following the injection of Dy-HMA, are all very small. At 24 hours with formulation B, the major site for the accumulation of leakage of activity is the liver, and this is only 0.003% of the injected dose. Formulation A gives 0.05% at 24 hours. For a patient this may be estimated to give less than 0.02 cGy exposure to the liver [Sledge *et al.* 1978]. Leakage to the other organs is even less. For each formulation, the leakage to the blood, kidney and urine is approximately the same at both 6 and 24 hours, but the accumulation in the liver decreases by at least an order of magnitude at 24 hours. Compared to formulation A, formulation B gives a lower leakage to all the tissues and a considerably reduced leakage to the liver at 24 hours. There does not appear to be any simple explanation for this difference and further studies are in progress to eliminate any error that may occur because of the small number of rabbits involved.

Compared to Dy-FHMA, Dy-HMA gives similar leakage to the lymph nodes at 6 and 24 hours. However, it must be noted that in the studies by Sledge *et al.* [1978] only one lymph node of each leg was measured whereas in the present studies the three regional lymph nodes were taken. Consequently, leakage to the individual nodes is probably much lower with Dy-HMA. Leakages to the blood and kidney are considerably less with Dy-HMA at both 6 and 24 hours. There is at least two orders of magnitude less for the blood and at least one order of magnitude for the kidney. At 24 hours, formulation B gives only about 0.3×10^{-3} per cent of the injected dose to the kidney. At six hours, leakage to the liver is approximately six times higher with both formulations of Dy-HMA. At 24 hours, leakage from formulation A is six times lower and formulation B is two orders of magnitude lower than with Dy-FHMA. Activity in the urine is an order of magnitude lower with Dy-HMA at both 6 and 24 hours.

Leakages with ^{90}Y silicate colloid are substantially higher than with Dy-HMA. The major site of accumulation with the colloid is again the liver, with 4.4 per cent of the injected dose occurring at 24 hours. The kidney also is very high with 0.33 per cent occurring at 24 hours. Compared to Dy-HMA at six hours, ^{90}Y leakage to the lymph nodes is 2.3 times higher; 10 times higher in the blood; 177 times higher in the kidney; 1.3 times higher in the liver and 318 times higher in the urine. At 24 hours, leakage to the lymph nodes is 5.9 times higher; 21 times higher in the blood; 1200 times higher in the kidney; 1250 times higher in the liver and 170 times higher in the urine. Leakage at 72 hours remains high with the liver still being the major site of accumulation with 0.3 per cent of the total injected dose.

8. CONCLUSIONS

In these studies, it has been demonstrated that a ^{165}Dy product, Dy-HMA, suitable for radiation synovectomy, can be readily prepared as a suspension of macroaggregated hydroxide particles in saline. The product has a Dy concentration of 6 mg mL^{-1} and a pH in the range 10.5-11.0. The majority of the

particles are in the 3-5 μm range and there is a negligible amount of particles less than 0.45 μm. Dy-HMA can be autoclaved and is stable for at least twice the time it takes for the ^{165}Dy to decay to unusable levels. The product can be prepared for use in all the major Australian cities. It is expected that for a 10-11 GBq dose for the knee joint, a patient in Sydney will receive a 1 mL injection and a patient in Perth will receive 5 mL.

The formulation for Dy-HMA has significant advantages over the use of Dy-FHMA, a product developed in the USA. The absence of iron not only simplifies the preparation and quality control but has also medical advantages. Leakage of activity to the blood, kidney, liver and urine are considerably less with Dy-HMA. Studies with rabbits have demonstrated that the leakage following the intra-articular injection of the knee joint is minimal. At 24 hours, leakage to the liver, which is the major site of accumulation, is about 0.003 per cent of the injected dose. It appears that the use of ^{165}Dy-HMA for radiation synovectomy has considerable advantages over the currently used product, ^{90}Y silicate colloid. It is now intended to proceed with clinical trials of Dy-HMA.

9. REFERENCES

Bellinger, J., Hetherington, E.L.R. [1976] - AAEC/M90.*

Blake, D.R., Hall, W.O., Bacon, P.A., Dieppe, P.A., Halliwell, B., Gutteridge, J.Mc. [1981] - *Lancet*, 2:1142-1144.

Bruce, D.W., Hietbrink, B.H., Dubois, K.P. [1963] - *Toxicol. Appl. Pharm.*, 5:750-759.

Davis, M.A., Jones, A.G., Trindade, H. [1974] - *J. Nucl. Med.*, 15(11)923-927.

Deckart, H., Tamaschke, J., Ett, S., Tantz, M., Knapp, G. [1979] - *Radiobiol. Radiother.*, 3:363-370.

Fleming A, Benn, R.T., Corbett, M., Wood, P.H. [1976] - *Ann. Rheum. Dis.*, 35:361-364.

Gumpel, J.M., Beer, T.C., Crawley, J.C.W., Farran, H.E.A. [1975] - *Br. J. Radiol.*, 48:377-381.

Haley, T.J. [1965] - *J. Pharm. Sci.*, 54(5)663-670.

Hnatowich, D.J., Kramer, R.I., Sledge, C.B., Noble, J., Shortkroff, S. [1978] - *J. Nucl. Med.*, 19(3)303-308.

Laurin, C.A., Desmarchais, J., Daziano, L., Gariysy, R., Derome, A. [1974] - *J. Bone Joint Surg.*, 56A:521-531.

Mughabghab, S.F. [1984] - Neutron Resonance Parameters and Thermal Neutron Cross Sections, Part B: Z=61-100. *Neutron Cross Sections*, Vol. 1. Academic Press, New York.

Noble, J., Jones, A.G., Davies, M.A., Sledge, C.B., Kramer, R.I., Livni, E. [1983] - *J. Bone Joint Surg.*, 65A:381-389.

Sekine, T., Baba, H. [1981] - *J. Inorg. Nucl. Chem.*, 43:1107.

Sledge, C.B., Atcher, R.W., Shortkroff, S., Anderson, R.J., Bloomer, W.D., Hurson, B. [1984] - *Clim. Orth.*, 182:37-40.

Sledge, C.B., Noble, J., Hnatowich, D.J., Kramer, R., Shortkroff, S. [1977] - *Arthritis Rheum.*, 20(7)1334-1341.

Sledge, C.B., Zuckerman, J.D., Zalutsky, M.R., Atcher, R.W., Shortkroff, S., Lionberger, D.R., Rose, H.A., Hurson, R.W., Lankenner, P.A., Anderson, R.J., Bloomer, W.A. [1986] - *Arthritis Rheum.* 29(2)153-159.

Sowby, F.D., ed. [1983] - Radionuclide Transformations. *ICRP Publication 38.* Pergamon Press, Oxford, pp.684-685.

* In April 1987, the Australian Atomic Energy Commission (AAEC) was replaced by the Australian Nuclear Science and Technology Organisation (ANSTO) which holds stocks of AAEC scientific reports and manuals.

Spooren, P.F.M.J., Rasker, J.J., Arens, R.P.J.H. [1986] - *Eur. J. Nucl. Med.*, 10:441-445.

Virkkunen, M., Krusius, F.E. Keiskarren, T. [1967] - *Acta Rheumatol. Scand.*, 13:81-91.

Urquhart, D.F. [1986] - AAEC/E627.*

Zalutsky, M.R., Venkatesan, P.P., English, R.J., Shortkroff, S., Sledge, C.B., Adelstein, S.J. [1986] - *Int. J. Nucl. Med. Biol.*, 12(6)457-465.

TABLE 1

COMPARISON OF ^{165}Dy AND ^{90}Y DOSES

Organ	Dy-FHMA* (cGy)	Y-Silicate Colloid (cGy)
Knee	9651	9160
Whole body	0.4	1.5 †
Ovaries	0.4	1.5 †
Liver	3.8	16.6 ††
Lymph node	16.6	3250**
		9100

*Zalutsky *et al.* [1986]; ** 10% leakage, Gumpel *et al.* [1975]; †Sledge *et al.* [1986]; †† 1.4% leakage.

TABLE 2

ACTIVITIES OF ^{166}Dy AND ^{166}Ho PRESENT IN AN 11 GBq THERAPY DOSE OF ^{165}Dy AT TIMES UP TO EIGHT HOURS EX-REACTOR FOR AN IRRADIATION OF EIGHT HOURS IN A THERMAL FLUX OF 5.0×10^{12} n cm $^{-2}$ s^{-1}

Time ex Reactor	Activity ^{166}Dy (MBq)		Activity ^{166}Ho (MBq)	
(h)	Calculated	Measured	Calculated	Measured
0	0.107	0.133	0.008	0.012
2	0.189	0.237	0.024	0.026
4	0.337	0.418	0.058	0.053
6	0.603	0.747	0.130	0.107
8	1.073	1.332	0.279	0.241

TABLE 3

RADIONUCLIDIC IMPURITIES IN IRRADIATED Dy_2O_3 AT TWO HOURS AND EIGHT HOURS EX-REACTOR FOR AN EIGHT-HOUR IRRADIATION IN A FLUX OF 5.0×10^{12} n cm^{-2} s^{-1}

Radionuclide	Percentage of total activity at given times	
	2 hours	8 hours
Dysprosium-157 (^{157}Dy)	0.0017	0.006
Dysprosium-166 (^{166}Dy)	0.0021	0.012
Holmium-166 (^{166}Ho)	0.0003	0.003
Samarium-153 (^{153}Sa)	6.3×10^{-5}	3.5×10^{-4}
Lutetium-177 (^{177}Lu)	0.0002	0.001
Lanthanum-140 (^{140}La)	9.0×10^{-7}	5.0×10^{-6}
Sodium-24 (^{24}Na)	1.7×10^{-5}	8.0×10^{-5}
Ytterbium-169 (^{169}Yb)	3.0×10^{-7}	1.8×10^{-6}
Ytterbium-175 (^{175}Yb)	9.6×10^{-6}	5.4×10^{-5}
Terbium-160 (^{160}Tb)	2.9×10^{-7}	1.7×10^{-6}
Gadolinium-153 (^{153}Gd)	6.0×10^{-8}	3.3×10^{-7}

TABLE 4

DYSPROSIUM MASS REQUIRED FOR THE INJECTION OF AN 11 GBq THERAPY DOSE OF ^{165}Dy FOLLOWING AN EIGHT HOUR IRRADIATION

Decay Time (h)	Mass* Dy (mg)	Mass** Dy (mg)
0	1.4	0.14
2	2.5	0.25
4	4.6	0.46
6	8.3	0.83
8	15.0	1.50
10	27.0	2.70

* Thermal flux of 5.0×10^{12} n cm^{-2} s^{-1}.
** Thermal flux of 5.0×10^{13} n cm^{-2} s^{-1}.

TABLE 5

RADIATION DOSES DUE TO ^{165}Dy AND THE IMPURITIES ^{166}Dy AND ^{166}Ho FOR AN 11 GBq INJECTION OF ^{165}Dy-FHMA TO THE KNEE FOR RADIATION SYNOVECTOMY

The calculations are for dysprosium irradiated for eight hours in a thermal flux of 5.0×10^{12} n cm^{-2} s^{-1} and injected two hours after the end of the irradiation.

Organ	Absorbed dose (cGy)			
	^{165}Dy*	^{166}Dy†	^{166}Ho†	^{166}Dy†
Knee	9650.6	1.28	5.65	6.93
Whole body	0.42	0.00058	0.00161	0.00219
Liver	3.82	0.00135	0.00811	0.00946
Lymph nodes	16.55	0.0917	0.9248	1.0165
Ovaries	0.02	0.000155	0.001353	0.00151

* Zalutsky *et al.* 1986; † this work.

TABLE 6

RADIATION DOSES DUE TO ^{165}Dy AND THE IMPURITIES ^{166}Dy-^{166}Ho FOR AN 11 GBq INJECTION OF ^{165}Dy FHMA TO THE KNEE FOR RADIATION SYNOVECTOMY

The calculations are for dysprosium irradiated for eight hours in a thermal flux of 5.0×10^{12} n cm^{-2} s^{-1} and injected eight hours after the end of the irradiation.

Organ	Absorbed dose (cGy)			
	^{165}Dy*	^{166}Dy†	^{166}Ho†	^{166}Dy + ^{166}Ho
Knee	9650.6	7.32	33.48	40.8
Whole body	0.42	0.00329	0.00929	0.01258
Liver	3.82	0.00769	0.04724	0.05493
Lymph nodes	16.55	0.522	5.293	5.815
Ovaries	0.02	0.000857	0.00778	0.00864

* Zalutsky *et al.* 1986 (5); † this work.

TABLE 7

PATIENT DOSE* Dy CORRECTED FOR TRANSPORT TIME TO AUSTRALIAN CITIES

City	Transport** Time (h)	Dy (mg)
Sydney	5	6
Melbourne	6.25	8.7
Brisbane	6.25	8.7
Adelaide	7	10.8
Perth	10	30.5

* 11 GBq; ** transport time = flight time + 5 h handling time.

TABLE 8

PERCENTAGE OF ACTIVITY ON PARTICLES OF Dy-HMA

%<12 µm	%<5 µm	%<3 µm	%<2 µm	%<1 µm	%<0.45 µm
99	58				<0.01
104	79				<0.01
101	65				<0.01
98	83				<0.01
99	82				<0.01
97	83			0.5	<0.01
99	79			0.7	<0.01
99	101				<0.01
98	99	0.7			<0.01
101	89	1.3	0.1		<0.01
99*	94				<0.01
97*	95				<0.01
89**	84	1.4	0.5		<0.01
90**	69	0.5	0.1		<0.01
92**	77	1.0			<0.01
94**	85	1.7			<0.01
88*†	74	0.5		<0.01	
89*†	71	0.6		<0.01	
98†	93				2
91††	85				1.4

* ^{153}Sm as tracer; ** Formulation B;

*† Formulation B autoclaved at 121°C for 33 min;

† Dy-FHMA, Hnatowich *et al.* [1978]; †† Dy-FHMA, Noble *et al.* [1983].

TABLE 9

EFFECT OF ULTRASONIC TREATMENT ON PARTICLE SIZE OF Dy-HMA

Treatment Time	%<12 μm	%<0.45 μm
0	81	0.05
10 s	76	<0.01
30 s	94	<0.01
45 s	94	<0.01
1 min	99	<0.01
2 min	97	<0.01
3 min	97	<0.01
4 min	97	<0.01

TABLE 10

EFFECT OF AUTOCLAVING ON PARTICLE SIZES OF Dy-HMA

Autoclave	Ultrasonic*	%<12 μm	%<5 μm	%<1 μm	%<0.45 μm
0	0	93			<0.01
0	0	93	83	0.2	<0.02
6 min	0	45	37	0.1	<0.01
6 min	0	69	52	0.1	<0.01
6 min	0	74	64	0.1	<0.01
6 min	1 min	94	87	0.2	<0.01
6 min	1 min	102			<0.01
6 min	1 min	98			<0.01

* Treatment only after autoclaving.

TABLE 11

EFFECT OF STANDING TIME ON PARTICLE SIZES OF Dy-HMA*

Time	%<12 µm	%<1 µm	%<0.45 µm
0.5 h	96	0.32	<0.02
2.0 h	97	0.50	<0.02
7.0 h	99	0.70	<0.02
24 h	94	0.31	<0.02
1.3 d	96	0.01	<0.01
2 d	95	0.02	<0.02
2.3 d	98	0.01	<0.01
9 d	97	0.03	<0.02
15 d	96	0.01	<0.01

* Formulation A, approx. 0.4 mg Dy mL^{-1}.

TABLE 12

EFFECT OF STANDING TIME ON PARTICLE SIZES OF Dy-HMA*

Time	%<12 µm	%<5 µm	%<3 µm	%<2 µm	%<0.45 µm
0	89	84	1.4	0.5	<0.01
0	88	70	0.5	0.1	<0.01
5 h	83	78	1.2	-	<0.01
1 d	90	77	0.4	-	<0.01
2 d	92	84	1.1	-	<0.01
3 d	94	88	1.8	-	<0.01

* Formulation B, approx. 6 mg Dy mL^{-1}.

TABLE 13

WASHOUT STUDIES OF Dy-HMA

Time	%<0.45 µm
0	0.006
3 h	0.010
6 h	0.016
24 h	0.009

TABLE 14

EFFECT OF THE AMOUNT OF Dy USED IN METHOD A

Dy* (mg)	% Precipitation	%<12 μm	%<5 μm	%<0.45 μm
0.4	80	90	82	< 0.01
1.1	79	87	86	0.02
2.1	89	99	98	< 0.01
3.2	87	96	96	< 0.01
4.2	86	98	97	< 0.01
8.4	87	99	95	< 0.01
12.6	73	98	96	0.4
16.8	55	83	82	12

* Total dysprosium before precipitation.

TABLE 15

EFFECT OF DILUTION ON PARTICLE SIZE

Dilution Factor	%<12 μm	%<5 μm	%<0.45 μm
1	89	82	<0.01
2	96	92	0.01
4	95	88	<0.02

TABLE 16

BIODISTRIBUTIONS AT 6 HOURS FOLLOWING INTRA-ARTICULAR INJECTION
(% of injected dose × 10^{-3})

Formulation	Lymph*	Blood**	Kidney*†	Liver†	Urine††
A		0.23	9	2.4	1.0
A		0.11	3.5	0.37	1.3
A		0.06	3.5	2.0	0.58
A		0.06	4.3	0.29	0.55
A		0.064	0.5	0.22	0.63
A		0.006	0.5	0.04	0.11
A		0.097	9.3	29	0.26
B	1	0.009	0.5	0.046	0.03
B	1.5	0.063	2.2	12.9	0.47
B	0.0	0.001	0.0	0.001	0.0

* Total of 3 regional lymph nodes; ** per mL; *† total right kidney; † per g; †† per 5 mL.

TABLE 17

BIODISTRIBUTIONS AT 24 HOURS FOLLOWING
INTRA-ARTICULAR INJECTION

(% injected dose x 10^{-3})

Formulation	Lymph*	Blood**	Kidney*†	Liver†	Urine††
A		0.11	5.5	0.40	0.52
A		0.07	9.0	0.56	0.70
A		0.007	8.0	0.94	0.25
A		0.042	2.3	0.36	0.22
A		0.012	0.75	0.05	0.12
A		0.03	8	1.00	0.84
B	1	0.006	0.5	0.068	0.14
B	0.0	0.015	0.0	0.012	0.21
B	3.5	0.006	0.3	0.026	0.33

* Total of 3 regional lymph nodes; ** per mL; *† total right kidney; † per g; †† per 5 mL.

TABLE 18

COMPARISON OF BIODISTRIBUTIONS BETWEEN Dy-HMA, Dy-FHMA AND ^{90}Y COLLOID (% injected dose $\times 10^{-3}$)

Formulation	Time	Rabbits	Lymph*	Blood**	Kidney***	Liver†	Urine††
A	6	7	-	0.090 (0.006-0.064)†††	4.4 (0.5-9.3)	4.9 (0.04-29)	0.63 (0.11-1.3)
B	6	3	0.83 (0.0-1.5)	0.024 (0.001-0.063)	0.9 (0.0-2.2)	4.3 (0.001-12.9)	0.17 (0.0-0.47)
H*†	5	7	1.0 (0.0-3.3)	17 (4-49)	27 (5-83)	0.7 (0.2-1.3)	3.0 (0.8-9.7)
Y*††	6	3	1.9 (1-4.5)	0.23 (0.0-0.44)	160 (123-209)	5.4 (3.5-7.0)	54 (18-80)
A	24	6		0.045 (0.007-0.11)	5.6 (0.75-9.0)	0.55 (0.05-1.0)	0.44 (0.12-0.84)
B	24	3	1.5 (0.0-3.5)	0.009 (0.006-0.015)	0.27 (0.0-0.5)	0.035 (0.012-0.068)	0.23 (0.14-0.33)
H**	24	5	3.0 (0.7-12)	34 (4-77)	140 (21-326)	3.7 (1.7-6.3)	44 (1-151)
Y††	24	3	8.9 (6-10)	0.19 (0.02-0.45)	332 (256-487)	44 (5-117)	40 (8-66)
Y*††	72	3	11.3 (0-21)	0.31 (0.0-0.93)	70 (45-90)	2.7 (1.6-3.3)	11 (0-21)

* Total of 3 regional lymph nodes; ** per mL; *** total right kidney;

† per g; †† per 5 mL; ††† range of values;

*† Dy-FHMA [Hnatowich *et al.* 1978]; *†† ^{90}Y silicate colloid.

APPENDIX A

COMPARISON OF ^{165}Dy ACTIVITIES DETERMINED BY CALIBRATED IONISATION CHAMBER AND BY GAMMA SPECTROMETRY

All activities have been corrected to a common reference time.

Sample details: 10 mL ^{165}Dy solution in standard thin walled counting vial.

Measured activity: (ionisation chamber) 8.56 GBq (± 10%)

Mean activity: (gamma spectrometer) 7.85 GBq (± 10%)

Photo Peak Energy (MeV)	^{165}Dy Activity (MBq)
0.2798	7.80
0.3617	7.66
0.6334	7.79
1.080	8.15