The in Vitro Characterization and Biostability of ^{99m}Tc-Dextran and Its Accumulation Within the Inflamed Paws of Adjuvant-Induced Arthritic Rats

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The *in vitro* and in vivo stability in normal and adjuvant-induced arthritic rats of ^{99m}Tc-dextrans (10, 40 and 500 kDa) have been investigated. The circulation half-lives were molecular weight dependent, with 10 and 40 kDa fractions being cleared relatively rapidly due to their ability to cross the glomerular basement membrane. The 500 kDa dextran was eliminated more slowly although 79% had been removed from the circulation 4 h post injection which probably was due to its degradation by dextranases and subsequent glomerular excretion. Dextran accumulation by the RES was found to be similar for all molecular weight preparations with no significant differences found. The sequestration of the dextrans by tissues of the RES (liver, spleen and lung) was independent of clearance rate. No differences were seen between normal and arthritic groups. Accumulation of the polymers by inflamed paws greatly exceeded that of normal paws for the 10 kDa (5-fold) and 500 kDa (6-fold) although no differences were seen with the 40 kDa dextran.

KEY WORDS: ^{99m}Tc-dextran; plasma and biostability in rats; RES uptake; targeting of inflammation; adjuvant-induced arthritis.

INTRODUCTION

A variety of drug delivery systems of a colloidal nature have been shown to accumulate in regions of inflammation following intravenous injection. These have included liposomes (1,2), lipid microspheres (3,4), polystyrene microspheres (5) and hydrophilic polymers (6). The latter study focused on polyvinylpyrrolidone (PVP) accumulation in the paws of adjuvant-induced arthritic rats when arthritic/normal paw ratios of between 1.6 and 7.5 were recorded for PVP molecular weights ranging from 10 to 360 kDa.

PVP, while satisfying the requirement of aqueous solubility, does not permit the opportunity for conjugation with a range of drug molecules and is also non-biodegradable. In contrast, dextran is biodegradable and the ease of covalent drug attachment makes it a suitable polymer candidate for targeted drug delivery. A number of dextran-NSAID conjugates have been previously described (7) and the possibility exists for forming drug-dextran-antibody complexes (8) to enhance target specificity.

The current study, however, has the objectives of: (i) examining the relationship between dextran molecular weight and its association with inflamed tissues in the adjuvant-induced arthritic rat model, and (ii) to assess whether such polymer accumulation is exclusively a function of molecular mass by comparing the dextran data with earlier results for PVP in the same animal model (6).

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MATERIALS AND METHODS

Materials

Dextrans (10 and 40 kDa) were purchased from Pharmacia (UK). Dextran (500 kDa) fluorescein isothiocyanate (FITC) - dextran (500 kDa) and stannous chloride (anhydrous) were from Sigma (UK). Filters (pore size 0.4 μM) were supplied by Nipon Millipore (Japan). ^{99m}Tc-Pertechnetate ($^{99m}TcO^{4-}$) was dispensed from a ^{99m}Tc generator (Amersham UK) by the Medical Physics Department, UWCM, Cardiff. Amberlite resin (IRA-400, standard grade), EDTA (disodium salt), hydrochloric acid (AnalaR) and sodium chloride were all from BDH (UK), and Sephacryl S-400 (superfine) was obtained from Pharmacia (UK).

Experimental Animals

Adjuvant polyarthritis was induced in rats using a method derived from that first described by Pearson (9). Briefly, female Sprague Dawley rats, weighing approximately 200 g at commencement of experiment, were injected intradermally, dorsally, at the base of the tail with a suspension of finely ground *Mycobacterium butyricum* in heavy mineral oil (approximately 2 mg in 200 μL per rat). The arthritis appeared between days 10–14 post injection. The paws were given an arthritic score of 0–5 depending on severity of inflammation and only rats with a grade of 3 or more per paw were used.

Radiolabelling of Dextrans

Dextran (10, 40 and 500 kDa) was labelled with ^{99m}Technetium following the method described by Ercan *et al.* (10). To 1 mL dextran solution (50 mg/mL) was added 500 μL stannous chloride ($SnCl_2$, 0.6 mg/mL double distilled degassed water and used immediately). The final concentration of the $SnCl_2$ is crucial for successful labelling of the polymer, since levels over 0.3 mg/mL would result in radiolabelling of the colloidal tin (11). The solution was mixed rapidly and filtered before adding 1 mL ^{99m}Tc (50–185 MBq/mL). It was then vortexed for 30 s and allowed to stand for 30 min at room temperature. Any free ^{99m}Tc was removed by mixing the labelled dextran with activated Amberlite IRA-400 resin. Activation of this dry powder was carried out by mixing with 0.1 M HCl, allowing the resin to settle and removing the acid. The resin was repeatedly (× 4) washed with 0.85% w/v NaCl, removing saline between washings. Finally ^{99m}Tc-dextran was added to the activated IRA and vortexed for 3 min before centrifuging for 10 min at 1000 RPM and retaining the ^{99m}Tc-dextran solution. Owing to the relatively short half-life of ^{99m}-Tc (6 h), radiolabelled dextran was used immediately after its preparation. The labelling efficiency for each preparation was calculated from the pre- and post-IRA

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standards taken at the time of labelling. Efficiency of labelling showed no dependence on dextran molecular weight with a mean value of 74.5 ± 9.2 (S.D, n = 22). This value falls within the range of 50–80% as reported by Wingarth and Strand (12) for 70, 90 and 500 kDa dextrans using the same procedure. Any unbound ^{99m}Tc was removed from the dextran prior to use by association with the IRA-400 resin.

Gel Permeation Chromatography

Characterisation of the ^{99m}Tc-dextran was by GPC analysis. A column (50 cm × 0.7 cm, 20ml volume) was packed with Sephacryl S-400 at 4°C and equilibrated in 0.85% w/v NaCl. A flow rate of 150 μL min^{-1} was maintained, 300 μL fractions were collected and radioactivity counted using an LKB Wallac 1282 gamma counter.

Radiolabelled dextran was eluted within the bed volume of the column (18.0, 15.6 and 7.2 mL for 10, 40 and 500 kDa ^{99m}Tc-dextran respectively) while the free ^{99m}Tc peak appeared at 26.4 mL. The presence of dextran in each fraction was determined by the anthrone method (13).

In Vitro Stability of ^{99m}Tc-Dextran

The *in vitro* stability of the ^{99m}-Tc-dextran was analysed by GPC following incubation at 37°C in rat, rabbit and human serum employing dilutions of 1:1.4, diluent:dextran preparation. Incubation times were selected to reflect the approximate time spent in the circulation following i.v. injection (14) and were 3 h for 10 and 40 kDa, and 20 h for 500 kDa dextran. The amount of radioactivity associated with the polymer was expressed as a percentage of the total activity eluted from the column and was used as a measure of ^{99m}Tc-dextran stability.

In Vivo Stability of ^{99m}Tc-Dextran (500 kDa)

The 500 kDa polymer was expected to persist in the circulation for the longest period after i.v. injection, thus facilitating any possible loss of radiolabel over time. Stability was therefore assessed in order to ensure that accumulation of activity in inflamed and/or other tissues was due to polymer and not any circulating free ^{99m}Tc. Lower molecular weight dextrans were not investigated in vivo from a stability point of view owing to their expected short circulation half-lives.

Three normal rats were injected i.v. (10 mg /kg) and blood samples (200 μL) withdrawn from the nicked tail-vein into 100 μL EDTA (0.2M) at 2, 60 and 120 min post injection. Samples were centrifuged for 15 min at 5000 RPM and 100 μL of plasma run on the Sephacryl S-400 column.

Blood Clearance and Tissue Distribution of ^{99m}Tc-Dextran

Blood clearance and tissue accumulation of dextrans were studied following i.v. injection (18 × 10^6 CPM in 200 μL) into both normal and adjuvant-induced arthritic rats. A 50 μL standard was retained in order to calculate the dose administered. The amounts of dextrans injected were 19.0 mg kg^{-1} (10 kDa), 18.5 mg kg^{-1} (40 kDa) and 10.5 mg kg^{-1} (500 kDa). ^{51}Cr-labelled rat erythrocytes were injected to act as a blood pool marker and the blood clearance and tissue distribution of ^{99m}Tc-dextran determined as previously described for PVA (6).

Statistics

All comparisons were made using a t-test or a two-way analysis of variance (ANOVA). $P < 0.5$ was considered significant.

RESULTS

In Vitro Stability of ^{99m}Tc-Dextran

For both 10 and 40 kDa dextrans there was no apparent loss of free ^{99m}Tc on incubation with rat serum as indicated by the absence of an activity peak. There were slight shifts to lower volumes on incubation suggesting that binding of blood components to the dextran molecules might be occurring. The high molecular weight dextran (500 kDa) was incubated in rat serum for 0, 3 and 20 h at 37°C before running aliquots of serum on the S-400 column. The control peak, i.e. no incubation appeared at 7.2 mL which moved to 9.6 mL for 0 h incubation and to 12.0 mL after the 3 h and 20 h incubation. No free technetium was detected and 93% of the activity was associated with the dextran peaks. This pattern suggests stability of the radiolabel but with a degradation of the 500 kDa dextran to smaller molecular weight fractions, each retaining the intact ^{99m}Tc label. The 12 mL elution volume probably represents a dextran fraction of approximately 250 kDa.

Given the apparent instability of the 500 kDa fraction in rat serum, the stability was examined in both rabbit and human serum to test whether the degradation was species specific. Identical stability profiles were observed with no evidence of free ^{99m}Tc loss. It should, however, be appreciated that small amounts of free ^{99m}Tc may become bound to the column and thus remain undetected. Any percentage of the applied activity not associated with the polymer was therefore assumed to represent free ^{99m}Tc (Table 1). However, levels of free ^{99m}Tc which ranged from 2–9% were considered to be acceptable for *in vivo* studies.

Table I. *In Vitro* Stability of ^{99m}Tc-Dextran

Dextran (kDa)	Incubation time (h)	% CPM associated with ^{99m}Tc-dextran	% CPM associated with Free ^{99m}Tc	Serum type
10	0	94	6	Rat
	3	97	3	
40	0	95	5	Rat
	3	95	5	
500	0	93	7	Rat
	3	93	7	
	20	93	7	
500	0	97	3	Rabbit
	3	91	9	
	20	92	8	
500	0	98	2	Human
	3	97	3	
	20	97	3	

In Vivo Stability of ^{99m}Tc-Dextran

The stability of the 500 kDa ^{99m}Tc-dextran following i.v. administration to rats was assessed in order to ensure that any uptake of the labelled polymer within inflamed and other tissues was not due to any circulating free ^{99m}Tc (see Table II). Since the radiolabel has previously been shown to remain stable *in vitro* but with some polymer degradation, then stability of the polymer itself was of particular interest. Lower molecular weight dextrans were not investigated for *in vivo* stability due to their expected short circulation half-lives.

Elution profiles of the blood samples taken from the three rats at 2, 60 and 120 min were very similar. Three peaks were observed: A at 10.5 mL, B at 17.9 mL and C (after 120 min only) at 27.6 mL. These correspond to 50 kDa dextran, 10 kDa dextran and free ^{99m}Tc respectively. The reduction in % activity associated with peak A and the corresponding increase in peak B with time, is further evidence of dextran degradation which is accompanied by release of free ^{99m}Tc (2.3% at 2 min, 3.7% at 60 min and 10.3% at 120 min). These results are similar to those reported by Ercan *et al.* (10) who tested the stability of ^{99m}Tc-dextran (82 kDa) in serum samples following i.v. administration in dogs.

Blood Clearance of ^{99m}Tc-Dextran

Blood clearance of all dextran preparations was by first order kinetics with clearance curves which were monoexponential. No discernible differences in normal and arthritic rats were observed. Circulation half-lives of 24.6 min (10 kDa) $n = 7$, 36.5 min (40 kDa) $n = 8$ and 102.3 min (500 kDa) $n = 10$ were determined.

Tissue Distribution of ^{99m}Tc-Dextran

Tissue accumulation of i.v. administered ^{99m}Tc-dextran is shown in Table III which focuses on arthritic paw and RES data. Values expressed per g tissue allowed direct comparison between normal and arthritic paws (Fig. 1) since it corrects for the greatly increased size of the inflamed limbs. In addition, both hepato- and splenomegaly were sometimes evident in arthritic animals so presentation of data per g tissue along with the use of ^{51}Cr erythrocytes ensured a true reflection of tissue accumulation of dextran.

Table II. *In Vivo* Stability of 500 kDa ^{99m}Tc-Dextran (Peak Elution Volume (mL) and % Peak Association)

Time post injection (min)	Peak	Rat I	Rat II	Rat III	Mean value
2	A	10.8 mL 82.1%	10.8 mL 79.9%	9.6 mL 86.4%	10.4 mL 82.8%
	B	18.0 mL 15.8%	18.0 mL 16.8%	16.8 mL 12.0%	17.6 mL 14.9%
60	A	10.8 mL 79.9%	10.8 mL 82.4%	10.8 mL 78.6%	10.8 mL 80.3%
	B	18.0 mL 16.2%	18.0 mL 14.6%	18.0 mL 17.3%	18.0 mL 16.0%
120	A	9.6 mL 54.6%	10.8 mL 70.7%	10.8 mL 69.6%	10.4 mL 65.0%
	B	18.0 mL 34.8%	16.8 mL 20.8%	19.2 mL 18.4%	18.0 mL 24.7%
	C	26.4 mL 9.2%	28.8 mL 2.3%	27.6 mL 4.6%	27.6 mL 5.4%

Mean Elution Volumes: Peak A = 10.5 mL ≡ Dextran 500 kDa; Peak B = 17.9 mL ≡ Dextran 10 kDa; Peak C = 27.6 mL ≡ Free ^{99m}Tc.

Uptake of 10 and 40 kDa dextran by the liver, spleen and lungs (indicative of the main sites of RES uptake) exceeded that of 500 kDa (ANOVA, $P < 0.01$) with between 5.8 and 12.5% accumulated per g of tissue (low molecular weight dextran) compared to only 2.4% for 500 kDa dextran. No differences in RES capture of ^{99m}Tc-dextran were seen between normal and arthritic rats (t-test, $P > 0.1$). This was also true for free ^{99m}Tc accumulation with <1% per g captured by tissues of the RES.

Accumulation of 10 and 500 kDa dextran by arthritic paws exceeded that by normal paws (t-test, $P < 0.001$) but no differences were seen with 40 kDa dextran (t-test, $P > 0.1$). Overall, however, more 40 kDa dextran was captured by the paws than 500 kDa, while the latter accumulated to a greater extent than 10 kDa dextran (ANOVA, $P < 0.01$). Almost 6 times as much 500 kDa dextran was taken up per g by arthritic paws than by normal paws with 5 times more 10 kDa dextran associated with inflamed than with normal tissues. As data had already been corrected for any increased blood volume in inflamed paws by using ^{51}Cr-RBC as a blood pool marker, the increased uptake was due to the inflammatory condition of the tissue rather than to the effects of increased blood flow. In all cases less than 1% of the injected dextran dose was taken up by the arthritic paws, with no differences seen between the molecular weight fractions. Levels of free ^{99m}Tc uptake by the paws was also less than 1% per g tissue, but arthritic paws captured 2.8 times as much activity as normal paws. This may be attributed to increased access to inflamed sites via extravasation.

DISCUSSION

Radiolabelling efficiencies of dextran using the method of Ercan *et al.* (10) were consistently good, averaging almost 75% over the range of molecular weight studied and provided preparations sufficiently radiolabelled to enable monitoring of their fate *in vivo*. Gel permeation chromatography was used to characterise the ^{99m}Tc-dextran preparations and showed that between 86 and 95% of the eluted activity was associated with the dextran peaks. Free ^{99m}Tc appeared to adhere to the column since only 1.5% recovery on average was observed. This was not, however, considered a problem when applying this technique to examining the integrity of the ^{99m}Tc radiolabel as a tracer for dextran under a variety of in vitro experimental conditions. Incubation in normal rat serum of all three ^{99m}Tc-dextran fractions produced no evidence of a free ^{99m}Tc peak and with 93–97% of activity associated with the dextran peak. The slight shift observed in the dextran peak to lower volumes on increasing the incubation time suggests molecular mass increases which may be attributed to the adsorption of serum protein. For the 500 kDa fraction, a degradation of the dextran molecule was observed to a fraction of approximately 250 kDa. Degrada-

Table III. Tissue Uptake of i.v. ^{99m}Tc-dextran (Mean % Accumulation of Total Dose ± SD)

Dextran (kDa)	RES		PAWS		PAW RATIO Arthritic/ Normal
	Normal	Arthritic	Normal	Arthritic	
10	17.73 ± 23.03	16.58† ± 16.03	0.08 ± 0.05	0.51* ± 0.22	6.4
40	16.59 ± 13.74	13.78† ± 12.70	0.41 ± 0.32	0.45† ± 0.21	1.1
500	11.93 ± 16.72	10.99† ± 13.49	0.11 ± 0.05	0.94* ± 0.34	8.5
^{99m}Tc	0.82 ± 0.82	1.30† ± 1.59	0.34 ± 0.23	1.57* ± 0.88	4.6

† Arthritic = Normal ($P > 0.1$).
* Arthritic > Normal ($P < 0.01$).

tion was also observed for this fraction when incubation was carried out in rabbit and human serum.

Further evidence of degradation of the 500 kDa dextran was observed following i.v. injection into rats when a second dextran peak occurred at an elution volume corresponding to a lower molecular mass. Only after 120 min incubation was there evidence of free ^{99m}Tc (5.4%). This, when added to that possibly bound to the column, would indicate approximately 10.3% of free ^{99m}Tc released 2 h after i.v. injection of ^{99m}Tc-dextran.

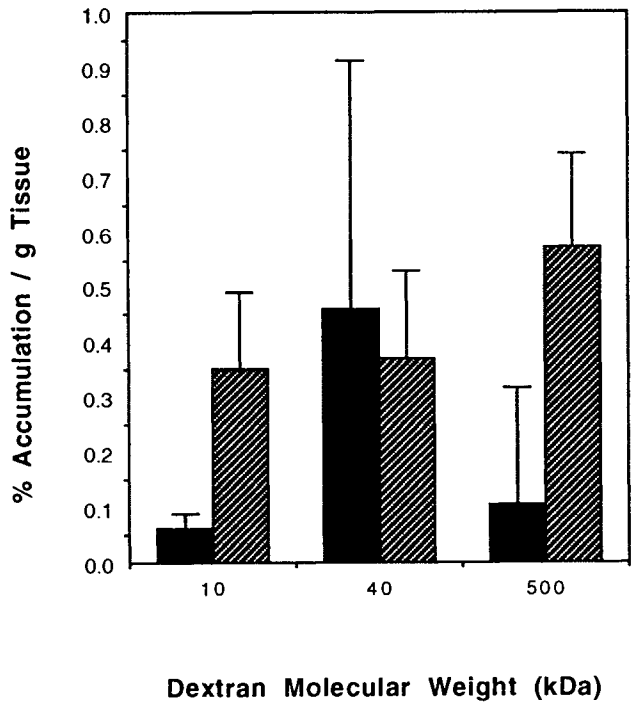

Fig. 1. Accumulation of i.v. ^{99m}Tc-dextran by normal (■) and arthritic (□) rat paws (mean % per g tissue ± SD. Total number of paws used (*n*) as follows:

Dextran kDa	Normal	Arthritic
10	12	12
40	12	11
500	20	12

These results are similar to those reported by Ercan *et al.* (10) who tested the stability of 80 kDa ^{99m}Tc-dextran in serum samples following i.v. administration in dogs from 5 min post injection. Initially only one dextran peak was observed following electrophoresis, but with increased circulation times up to three dextran peaks were found corresponding to the parent molecule and lower molecular weight fractions of metabolised dextran. As observed in the current studies, there was no evidence of a free ^{99m}Tc peak, and integrity of the radiolabel was further confirmed by the absence of any thyroid or stomach activity. The *in vitro* stability of all dextran preparations was therefore found to be satisfactory as a maximum of only 9% free ^{99m}Tc was released after 20 h incubation in rat, rabbit and human serum. On average 4.8% free ^{99m}Tc was lost from low molecular weight (10 and 40 kDa) preparations and 5.4% from 500 kDa dextran when incubated in normal rat serum at 37°C (maximum 3 h and 20 h for low and high molecular mass dextran respectively).

The 500 kDa dextran exhibited complete radiolabel stability in vitro but some degradation of the polysaccharide was evident by a shift in the dextran-associated activity peaks towards those typical for lower molecular weight dextrans (from 7.2 mL to 12.0 mL). Levels of degradation which were apparent at 3 h incubation did not increase up to 20 h *in vitro*, and were probably due to serum enzyme activity. This was not a species specific effect as the pattern of stability with rat serum was repeated when rabbit and human serum were used.

The *in vivo* stability of the 500 kDa dextran, as determined following i.v. injection into three normal rats was less convincing than that seen *in vitro*. After only 2 min almost 15% of the dextran-associated activity appeared at an elution volume comparable to that achieved with 10 kDa dextran. By 1 h post injection, the amount of activity presumably associated with a 10 kDa dextran fraction had increased to 16.0% which, after 2 h, increased to nearly 25% of the activity recovered. Release of free ^{99m}Tc from the complex was also evident with levels increasing from 2.3% (2 min)

and 3.7% (1 h) to 10.3% at 2 h post injection. Even though these levels would appear to be negligible in comparison with the amount of dextran-associated intact radiolabel, it was considered necessary to carry out a control i.v. injection of free ^{99m}Tc as part of the *in vivo* distribution studies to assess its tissue accumulation and in order to correct for any possible loss of label from ^{99m}Tc-dextran.

The increased levels of dextran degradation seen *in vivo* compared with *in vitro* were probably due to levels of dextranases in the liver which the dextran would encounter almost immediately on i.v. injection, with so-called first pass metabolism occurring as a result (15). With increased circulation times there would be prolonged contact with such enzymes leading to a progressive degradation of the dextran molecule, a phenomenon which has been shown to occur *in vivo*. Mehvar and Shepard (16) have determined the average molecular weight of dextrans excreted in urine following i.v. injection of FITC-dextran in rats. Molecular weights of the excreted polymers were fairly constant (26, 33 and 25 kDa) after injection of 40, 70 and 150 kDa dextrans respectively, while those of the lower molecular weight preparations (20 and 4 kDa) were largely unaltered in the excreted form (18 and 3 kDa). Thus high molecular weight dextrans (> 40 kDa) which are unable to traverse the glomerular membrane for direct excretion via the kidney are eventually eliminated following degradation to smaller fractions. Therefore one of the properties of dextran which make it so suitable as a prospective drug carrier, i.e., its biodegradability, may also prove to be an obstacle in limiting its circulating half-life and thus reducing the opportunities for tissue capture.

Half-life values for low molecular weight dextrans following i.v. injection in normal and arthritic rats (24.6 min, 10 kDa and 36.5 min, 40 kDa) were comparable with those reported throughout the literature, but 500 kDa dextran was cleared more rapidly than expected (102.3 min) when compared to circulation half-lives reported for lower molecular weight fractions (70, 82 and 150 kDa). Arturson and Wallenius (14) have described the i.v. injection of dextran (average molecular weight 40 kDa, range 10–80 kDa) in normal humans, with blood clearance expressed as a percentage of the initial serum dextran concentration. They found that dextrans in the molecular weight ranges 14–18 kDa and 36–44 kDa had half-lives of 15 min and 65 min respectively. These values are similar to those of our 10 and 40 kDa dextran preparations (in rat), which had half-lives of 25 and 37 min respectively. However, Melton *et al.* (17) have demonstrated a half-life of 15.3 h for 40 kDa dextran given i.v. to mice. Molteni (18) has reported that i.v. administered dextrans of 110 kDa can circulate for 2–3 days, while 70 kDa has 40 % remaining in the circulation after 24 h.

Dextrans below 40 kDa are completely excreted in the urine within 48 h, while 80 % of 40 kDa dextran has been excreted 6 h after i.v. injection (18). Our results concur with these findings for 40 kDa dextran, where 86% of the injected activity had been lost from the circulation within 2 h. However, Arturson and Wallenius (14) have also shown that dextrans of between 55 and 69 kDa exhibited half-lives of over 12 h. Mice given i.v. dextran (40, 70 110 and 150 kDa) exhibited circulation half-lives of between 15 and 19 h (17). Our half-life value of 1.7 h with 500 kDa dextran clearly deviates considerably from these results and could not be explained simply by the different species used. However, in a recent study of the pharmacokinetics of i.v. administered FITC-dextran in rats, Mehvar and Shepard (16) have found similar clearance profiles to those reported here. Terminal half-lives of FITC-dextrans of molecular weights 20, 40 and 150 kDa were 0.5h, 1.9h and 3.0 h respectively which more closely resemble our results using parent dextrans in the same species. FITC-dextrans of 40 kDa and above could be detected in serum up to 12 h after dosing while a 20 kDa preparation had been cleared after only 3 h.

The possibility of an unstable ^{99m}Tc-dextran preparation resulting in the problems encountered was ruled out by the use of an i.v. injection of commercially produced FITC-dextran (500 kDa) with blood clearance investigated in four normal rats of the same strain utilised throughout the study. A circulating half-life of 24.2 min was observed which more closely resembled that seen for 10 kDa ^{99m}Tc-dextran (24.6 min) than for the 500 kDa dextran preparation. This in itself may be explained by the fact that anionic FITC-dextran is cleared more rapidly from the bloodstream than parent dextran (19).

The uptake of 10 and 40 kDa ^{99m}Tc-dextran into the liver, spleen and lungs of both normal and arthritic rats exceeded that of the 500 kDa complex (ANOVA, $P < 0.01$). These differences may be explained by taking into account circulating levels of each dextran preparation at the time of sacrifice; percentage of injected polymer still in the blood stream corresponded to 12.5%, 14.3% and 20.9% (10, 40 and 500 kDa respectively) therefore the high molecular mass dextran had not been cleared to such an extent as the two lower molecular weight preparations, with the RES being responsible for much of the observed clearance. No differences in RES capture of ^{99m}Tc-dextran were seen between normal and arthritic rats (*t*-test, $P > 0.1$). This was also true for free ^{99m}Tc accumulation with <1% per g captured by tissues of the RES.

Tissue uptake of dextran by normal and arthritic rat paws is given in Table III and Figure 1. Accumulation of 10 and 500 kDa dextran by arthritic paws exceeded that by normal paws (*t*-test, $P < 0.001$), but no differences were seen with 40 kDa dextran (*t*-test, $P > 0.1$). The latter may result from an unusually high mean and S.D. for the normal joints (0.47 ± 0.45). Overall however, more 40 kDa dextran was captured by the paws than 500 kDa, while the latter accumulated to a greater extent than 10 kDa dextran (ANOVA, $P < 0.01$). Almost 6 times as much 500 kDa dextran was taken up per g by arthritic paws than by normal paws, with 5 times more 10 kDa dextran associated with inflamed than with normal tissues. When expressed as a percentage of the total dose administered, 8.5 times as much 500 kDa dextran was captured by arthritic than by normal paws, and 6.4 times as much by inflamed paws of the 10 kDa dextran preparation. As data had already been corrected for any increased blood volume in inflamed paws by using ^{51}Cr-RBC as a blood pool marker, the increased uptake was due to the inflammatory condition of the tissue rather than to the effects of increased blood volume. In all cases less than 1% of the injected dextran dose was taken up by the arthritic paws, with no differences seen between the molecular weight groups. Levels of free ^{99m}Tc uptake by the paws was also less than 1% per g tissue, but arthritic paws captured 2.8 times as much activity

as normal paws. This may be attributed to increased access to inflamed sites via extravasation, and was not thought to make any considerable contribution to paw uptake of the polymer where activities were accumulated at up to 5.7 times as much by arthritic as by normal paws.

It is still unclear whether the increased paw uptake seen here is entirely due to escape of dextran at sites of inflammation as a result of such increases in capillary permeability (i.e., passive uptake), or whether it is due to the endocytic capture of the polymer by infiltrating and resident phagocytes (active targeting). Most probably the phenomenon is a result of a combination of the two effects. Accumulation of the polymer may be different when drug is covalently bonded, a process which will alter the elimination rate from the blood pool.

Comparing uptake of dextran with previously published data for PVP, 3 times as much 360 kDa PVP was taken up by arthritic paws as 500 kDa dextran. This increase was probably due to the inflamed tissues continuing to accumulate high molecular weight PVP over the 28 h experimental period, while 500 kDa dextran, being hydrolysed and thus rapidly eliminated from the circulation, was not available for similar levels of uptake. The accumulation data reported in this paper for the 500 kDa dextran fraction may, however, be due in part to uptake of the lower molecular mass dextrans formed as a result of hydrolysis. However, almost twice as much 10 kDa dextran as PVP of the same molecular weight was associated with arthritic paws following i.v. injection (% per g tissue), despite the high levels of RES capture seen.

The amount of 360 kDa PVP captured by inflamed tissues (3.6% total dose) exceeded that of 80 nm polystyrene microspheres as reported by Illum *et al.* (5), where less than 1% of the total dose administered i.v. to rabbits was located at the site of inflammation 24 h post injection. This figure compares with 500 kDa dextran, i.e., 0.9% total dose accumulated after 4 h. When the microspheres were coated with Poloxamine 908, a procedure which leads to their retention within the general circulation, more than 10% of the dose reached the inflammation site which the authors tentatively attributed to some active transport process (5).

REFERENCES

1. W.G.Love, N.Amos, I.W.Kellaway, B.D.Williams. Specific accumulation of technetium-99m radiolabelled negative liposomes in the inflamed paws of rats with adjuvant induced arthritis: effect of liposome size. *Ann. Rheum. Dis.* 48, 143-148. 1989.
2. W.G.Love, N.Amos, I.W.Kellaway, B.D.Williams. Specific accumulation of cholesterol-rich liposomes in the inflammatory tissue of rats with adjuvant arthritis. *Ann Rheum. Dis.* 49, 611–614. 1990.
3. Y.Mizushima. Lipid microspheres as novel drug carriers. *Drugs Exptl. Clin. Res.* 11, 595–600. 1985.
4. Y.Mizushima, Y.Wada, Y.Etoh, K.Watanabe. Anti-inflammatory effects of indomethacin incorporated in a lipid microsphere. *J. Pharm. Pharmacol.* 35, 398–399. 1983.
5. L.Illum, J.Wright, S.S.Davis. Targeting of microspheres to sites of inflammation. *Int. J. Pharm.* 52, 221–224. 1989.
6. L.Seale, W.G.Love, N.Amos, B.D.Williams, I.W.Kellaway. Accumulation of polyvinylpyrrolidone within the inflamed paws of adjuvant-induced arthritic rats. *J. Pharm. Pharmacol.* 44, 10–14. 1992.
7. C.Larsen, M.Johansen. Macromolecular prodrugs: XI Regeneration rates of various NSAID compounds from their corresponding dextran ester prodrugs in aqueous buffer and in different biological media. *Acta Pharm. Nordica* 2, 57–66. 1989.
8. K.Sheldon, A.Marks, R.Baumal. Sensitivity of multidrug resistant KB-C1 cells to an antibody-dextran-adriamycin conjugate. *Anticancer Research* 9, 637–642. 1989.
9. C.M.PEARSON. Development of arthritis, periarthritis and periostitis in rats given adjuvants. *Proc. Soc. Exp. Biol. Med. (NY).* 91, 95–101. 1956.
10. M.T.Ercan, M.Schneidereit, R.Senekowitsch, H.Kriegel. Evaluation of ^{99m}-Tc-dextran as a lymphoscintigraphic agent. *Eur. J. Nucl. Med.* 11, 80–84. 1985.
11. S.J.Farr, I.W.Kellaway, D.R.Parry-Jones, S.G.Woolfrey. ^{99m}Technetium as a marker of liposomal deposition and clearance in the human lung. *Int. J. Pharm.* 26, 303–316. 1985.
12. K.Wingardh, S.E.Strand. Evaluation in vitro and in vivo of two labelling techniques of different ^{99m}-Tc-dextrans for lymphoscintigraphy. *Eur. J. Nucl. Med.* 15, 146–151. 1989.
13. T.A.Scott, E.H.Melvin. Determination of dextran with anthrone. *Anal. Chem.* 25, 1656–1661. 1953.
14. G.Arturson, G.Wallenius. The intravascular persistence of dextran of different molecular sizes in normal humans. *Scand. J. Clin. Lab. Invest.* 1, 76–80. 1964.
15. C.Larsen. Dextran prodrugs—structure and stability in relation to therapeutic activity. *Adv. Drug Deliv. Rev.* 3, 103–154. 1989.
16. R.Mehvar, T.L.Shepard. Molecular weight dependent pharmacokinetics of fluorescein-labelled dextrans in rats. *J. Pharm. Sci.* 81, 908–912. 1992.
17. R.G.Melton, C.N.Wiblin, R.C.Foster, R.F.Sherwood. Covalent linkage of carboxypeptidase G_2 to soluble dextrans - I. Properties of conjugates and effects on plasma persistence in mice. *Biochem. Pharmacol.* 36, 105–112. 1987.
18. L.MOLTENI. Dextrans as drug carriers. In: *Drug Carriers in Biology and Medicine*. G.Gregoriadis, Ed., Academic Press. pp. 107–125. 1979.
19. B.H.Persson. Histochemical studies on the fate of parenterally administered dextran in rabbits. 2: On the accumulation of dextran within the kidney, liver, leucocytes and reticulo-endothelial system. *Acta Soc. Med. Ups.* 57, 421–437. 1952.