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# Accumulation of Polyvinylpyrrolidone within the Inflamed Paws of Adjuvant-induced Arthritic Rats

L. SEALE, W. G. LOVE, N. AMOS*, B. D. WILLIAMS* AND I. W. KELLAWAY

*Welsh School of Pharmacy, University of Wales College of Cardiff, Cardiff CF1 3XF and *Rheumatology Department, University of Wales College of Medicine, Cardiff CF4 4XW, UK*

**Abstract**—$^{125}$I-Labelled polyvinylpyrrolidone ([$^{125}$I]PVP) of a range of molecular weights (mol. wt 10, 40 and 360 kDa) was injected i.v. into adjuvant-induced arthritic and normal rats and the blood clearance and tissue distribution of the polymers determined. The half-life of PVP in the circulation increased with increasing mol. wt; 10, 40 and 360 kDa polymers had mean terminal half-lives of 2·2, 6·9 and 16·4 h, respectively. Tissue uptake was also found to be mol. wt dependent, the largest PVP molecule accumulating to a greater extent in the spleen, liver, lungs and paws in both normal and arthritic rats ($P < 0.01$) than the two lower mol. wt polymers. Accumulation of the polymer in inflamed paws (g tissue)$^{-1}$ greatly exceeded that of normal paws ($P < 0.01$). This difference was particularly noticeable with 360 kDa PVP, where arthritic paws amassed 7 times more PVP than normal paws.

Since most pharmacological agents are of low mol. wt, following oral administration they are distributed throughout the body rather than solely at the site of action and are often rapidly excreted (Ringsdorf 1975). Consequently, large and repeated doses must be given to be effective, often resulting in a range of deleterious side effects (Duncan & Kopecek 1984). A classic example is the gastrointestinal bleeding and ulceration caused by conventional anti-inflammatory therapy.

By attaching drugs to macromolecular carriers their half-life in the body can be increased, enabling lower doses of the drug to be used and thus minimizing toxic side effects (Duncan 1985). In addition, selective delivery of drugs to tissues where they exert their pharmacological effects will also enhance the desired therapeutic result.

Following i.v. administration, foreign particles are sequestered by the reticuloendothelial system within phagocytic cells distributed in the liver, spleen, lungs, lymph nodes and bone marrow (Altura 1980). Rheumatoid arthritis is characterized by chronic inflammation of the synovial membrane, local accumulation of phagocytic cells and increased permeability of the synovial blood vessels. It is proposed that a combination of these factors may lead to accumulation of i.v. administered polymers at sites of inflammation.

Previously, i.v. administered liposomes have been used to distinguish active rheumatoid disease (Williams et al 1986), and liposome-entrapped steroids have been administered intra-articularly to arthritic rabbits with favourable results (Dingle et al 1978). Mizushima (1985) has shown that i.v. injected lipid microspheres containing corticosteroid accumulate in inflamed rat paws, while indomethacin incorporated in the same carrier was 5 times more potent than the free drug (Mizushima et al 1983).

The aims of this study were to investigate the potential use of high mol. wt polymer-drug conjugates in the treatment of inflammatory disease such as rheumatoid arthritis. At present a number of candidate polymers are being investigated although this paper discusses the use of a neutral polymer, polyvinylpyrrolidone (PVP). As a non-biodegradable inert polymer, PVP has previously been used as a plasma expander, and its fate in the body after i.v. administration has been studied (Ravin et al 1952). Specific uptake by the reticuloendothelial system (Regoeczi 1976) has led to it being used as an indicator of the clearance function of macrophages (Morgan & Soothill 1975). Therefore, PVP has been used to characterize the effect of molecular mass on blood clearance and tissue distribution of i.v. injected polymers. Inflamed tissue was of particular interest and the extent of PVP (the potential drug carrier molecule) accumulation in such tissues was determined using an adjuvant-induced arthritic rat model of inflammation.

However, before any in-vivo studies of PVP distribution could be undertaken, a satisfactory method for radiolabelling the polymer had to be developed and stability of the radiolabel under in-vitro experimental conditions assessed. If PVP can then be shown to accumulate in inflamed tissues it may be possible to use the polymer for the delivery of pharmacologically active materials to these sites.

Correspondence: I. W. Kellaway, Welsh School of Pharmacy, UWCC, PO Box 13, Cardiff CF1 3XF, UK.

## Materials and Methods

PVP was purchased from Sigma (UK). Sodium iodide ($^{125}$I) and sodium chromate ($^{51}$Cr) were obtained from Amersham Radiochemicals (UK). Heavy mineral oil and Sephacryl S-400 were purchased from Sigma (UK) and Biogel P-60 from Biorad (UK). All other chemicals were purchased from BDH (UK) and were used as received. Sprague-Dawley rats were supplied by Tenovus Institute, University of Wales College of Medicine, Cardiff. *Mycobacterium butyricum* was obtained from Difco Laboratories (Detroit, USA).

### *Radiolabelling of PVP*

PVPs of mol. wts 10, 40 and 360 kDa were labelled with $^{125}$I using a method adapted from that used by Regoeczi (1976). PVP (50–100 mg) was dissolved in 1 mL sulphuric acid (0·2 M) at 0°C, and 0·1 mL of 10% w/v sodium nitrite and 500 $\mu$Ci sodium iodide (Na$^{125}$I) added. The mixture was irradiated

clotting. Subsequent blood samples were monitored for $^{125}I$ activity using the gamma counter to assess the approximate 0·85% w/v) to remove excess free iodide. Finally, the labelled PVP was neutralized with KOH (0·2 M) and reduced by adding 0·2 mL sodium sulphite (50 mg $mL^{-1}$).

*Gel permeation chromatography*

A Biogel P-60 column was used for the gel permeation chromatography (GPC) analysis of 10 and 40 kDa PVP and a Sephacryl S-400 column for 360 kDa PVP. Columns (50 × 0·7 cm, 20 mL volume) were packed at 4°C and equilibrated in 0·85% NaCl. Flow rates of 60 μL $min^{-1}$ for P-60 and 150 μL $min^{-1}$ for S-400 were used and sample volumes did not exceed 300 μL. Samples of iodinated PVP were analysed on the appropriate columns; PVP of mol. wt 360 kDa appeared within the fractionation range of Sephacryl S-400 (10–2000 kDa) while 40 and 10 kDa PVP were fractionated on Biogel P-60 (range 3–60 kDa). Fractions were collected and radioactivity counted using an LKB Wallac 1282 gamma counter.

*In-vitro stability of [$^{125}I$]PVP*

The stability of each mol. wt of [$^{125}I$]PVP was analysed by GPC. Samples of radiolabelled polymer were incubated in saline and normal rat serum (1 : 1·4, diluent : PVP) at 37°C for various times and analysed on the appropriate chromatography column. Time of incubation was chosen to reflect the approximate time spent in the circulation, estimated from PVP blood clearance data in the literature (Ravin et al 1952). Incubation times were 3 h for 10 and 40 kDa PVP, and 20 h for 360 kDa PVP. 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 the stability of [$^{125}I$]PVP.

*Adjuvant-induced arthritis in the rat*

Adjuvant polyarthritis in rats was induced by the method of Pearson (1956). Female Sprague-Dawley rats, each weighing approximately 200 g, were injected intradermally at the base of the tail with a suspension of finely ground *Mycobacterium butyricum* in heavy mineral oil (approx. 2 mg in 200 μL per rat). Onset of arthritis was between days 10–14 post-injection. Paws were given an arthritic score of 0–5, depending on the severity of inflammation (Trentham et al 1977), and only rats with a score of 3 or more per paw were used.

*In-vivo administration of [$^{125}I$]PVP*

Normal and arthritic rats (3–5 rats in each group) were used for each PVP preparation. Rats were anaesthetized with diethyl ether before injection of [$^{125}I$]PVP via the tail vein. To effectively monitor the fate of the polymer, approximately $1 \times 10^6$ counts $min^{-1}$ (0·45 μCi) in 250 μL was given to each rat, and a 20 μL standard retained for gamma counting in order to calculate the exact dose administered. The amount of polymer injected corresponded to 37·1 mg $kg^{-1}$ (10 kDa), 6·6 mg $kg^{-1}$ (40 kDa) and 5·8 mg $kg^{-1}$ (360 kDa). At the same time $^{51}Cr$-labelled rat erythrocytes ([$^{51}Cr$]RBC) were injected to act as a blood pool marker (Gray & Sterling 1950). Blood samples (50 μL) were collected at intervals from the nicked tail vein into a capillary pipette and immediately placed in 0·2 M ethylenediamine tetra-acetic acid (EDTA) to prevent with an ultraviolet lamp at 254 nm for 1 h in a quartz cuvette and then dialysed for 48 h against physiological saline (NaCl, clearance profiles. When approximately 80% of the injected activity had been cleared from the circulation, the rats were killed and the relevant tissues (including paws) removed and weighed. The amounts of [$^{125}I$]PVP and [$^{51}Cr$]RBC present in the tissues were quantified by gamma counting and corrections made for crossover between the two channels.

*Blood clearance calculations*

Percentage of the initial PVP dose remaining in the circulation was plotted against time post injection and a line of best fit obtained. Clearance curves for all PVP preparations were biexponential. The circulating half-lives (initial and terminal) of PVP were calculated from the corresponding elimination constants (k) from the formula:

$$\text{half-life} = \frac{0{\cdot}693}{k} \quad (1)$$

*Tissue uptake calculations*

In order to calculate the percentage accumulation of [$^{125}I$]PVP by each tissue, the dose of [$^{125}I$]PVP given to each rat (A) was calculated from the standard. The final blood sample, taken at the time of death, was counted for [$^{125}I$]PVP (B) and [$^{51}Cr$]RBC (C), since these values will be reflected in tissue counts. The ratio of these is B/C. Then:

$$\%\ \text{total accumulation} = \text{tissue } {}^{125}I - \frac{(\text{tissue } {}^{51}Cr \times B/C) \times 100}{A} \quad (2)$$

$$\%\ \text{accumulation (g tissue)}^{-1} = \frac{\%\ \text{total}}{\text{wt in g}} \quad (3)$$

Uptake values were expressed as a percentage (g tissue)$^{-1}$. This takes into account the greatly increased size of inflamed paws.

*Statistics*

All comparisons were made using analysis of variance. $P < 0{\cdot}05$ was considered significant.

## Results

*In-vitro stability of [$^{125}I$]PVP*

Following incubation in physiological saline and normal rat serum at 37°C, iodinated PVP preparations (10, 40 and 360 kDa) were relatively stable (Table 1). Clearly incubation in saline had no effect on the [$^{125}I$]PVP since more than 97% of the activity eluted from the GPC column following incubation was found to be associated with the polymer. Minimal dissociation of the radio-isotope was seen after a 3 h

Table 1. In-vitro stability of [$^{125}I$]PVP.

| PVP mol. wt (kDa) | Incubation time (h) | Percentage $^{125}I$-associated PVP following incubation at 37°C | |
|---|---|---|---|
| | | Saline | Normal rat serum |
| 10 | 3 | 97 | 96 |
| 40 | 3 | 99 | 97 |
| 360 | 7 | 97 | 93 |
| 360 | 20 | 97 | 86 |

incubation of 10 and 40 kDa [$^{125}$I]PVP in rat serum, with 4 and 3% free iodide released, respectively. However, after incubation of the 360 kDa [$^{125}$I]PVP preparation with rat serum for 20 h, 14% free iodide had been released from the polymer.

*Blood clearance of i.v. [$^{125}$I]PVP*

Blood clearance profiles of 10 and 40 kDa [$^{125}$I]PVP are shown in Fig. 1 and that of 360 kDa PVP in Fig. 2 (mean values ± s.d., normal and arthritic). The clearance curves of the polymers exhibited biphasic first order kinetics for all mol. wt PVPs (Table 2). Rate constants (k) for each clearance phase were calculated from the graphs and half-lives determined as previously described. For 10, 40 and 360 kDa PVP respectively, initial half-lives were 6·1, 6·9 and 35·6 min while terminal half-life values were 2·2, 6·9 and 16·4 h (mean values for normal and arthritic rats, n = 7 or 8 for each mol. wt group). Thus the half-life of PVP is dependent upon its mol. wt, with the 10 kDa PVP being almost totally cleared 2·5 h after injection, while a considerable proportion of the 40 and 360 kDa PVP remains in the bloodstream at this time. Blood clearance data for normal and arthritic rat groups showed no significant differences for 10 and 40 kDa PVP ($P > 0·1$), but clearance of 360 kDa PVP by arthritic rats exceeded that of

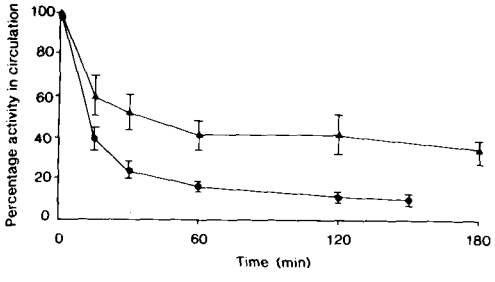


FIG. 1. Blood clearance of i.v. [$^{125}$I]PVP (10, ● and 40 kDa, ▲) in normal and arthritic rats (pooled data, mean ± s.d.).

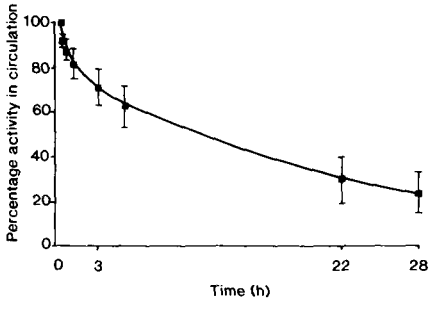


FIG. 2. Blood clearance of i.v. [$^{125}$I]PVP (■, 360 kDa) in normal and arthritic rats (pooled data, mean ± s.d.).

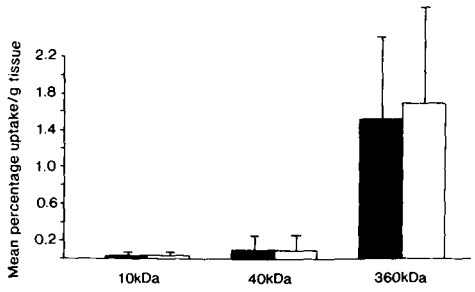


FIG. 3. Accumulation of i.v. [$^{125}$I]PVP by the reticuloendothelial system of normal (■) and arthritic rats (□) (mean ± s.d.).

the normal animals at 5, 22 and 28 h post-injection ($P < 0·01$). However, for the purpose of this study—to determine the effect of mol. wt on distribution of polymers—mean values of normal and arthritic rats combined were used.

*Tissue distribution of i.v. [$^{125}$I]PVP*

Accumulation of [$^{125}$I]PVP by tissues of the reticuloendothelial system is shown in Fig. 3. Data for liver, spleen and lungs were used as indicative of the main sites of uptake. Uptake of 360 kDa PVP exceeded that of the two lower mol. wt preparations ($P < 0·01$). Up to 1·7% of the injected 360 kDa PVP was accumulated (g tissue)$^{-1}$ whereas the accumulation of the 40 and 10 kDa preparations was less than 0·1% (g tissue)$^{-1}$. No differences were seen between normal and arthritic groups ($P > 0·1$), except in the liver where uptake of 360 kDa PVP only was greater in arthritic than in normal rats ($P < 0·01$).

Tissue uptake data for normal and arthritic paws is shown in Fig. 4. Accumulation of PVP by arthritic paws exceeded that by normal paws for all mol. wt preparations ($P < 0·01$). In addition, a mol. wt correlation was seen in arthritic paws; uptake of PVP 360 > 40 > 10 kDa ($P < 0·01$), compared with

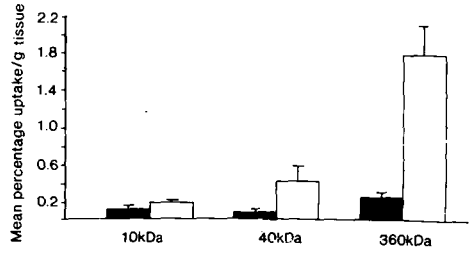


FIG. 4. Accumulation of i.v. [$^{125}$I]PVP by normal (■) and arthritic rat paws (□) (mean ± s.d.).

Table 2. Blood clearance of i.v. [$^{125}$I]PVP (mean ± s.d.).

| | Half-life | | | | | |
|---|---|---|---|---|---|---|
| | Initial (min) | | | Terminal (h) | | |
| PVP mol. wt (kDa) | Normal | Arthritic | Mean | Normal | Arthritic | Mean |
| 10 | 6·5 ± 1·7 | 5·9# ± 1·9 | 6·1 ± 1·8 | 2·1 ± 0·8 | 2·2# ± 0·9 | 2·2 ± 0·8 |
| 40 | 5·9 ± 3·1 | 7·6# ± 2·2 | 6·9 ± 2·5 | 8·3 ± 3·7 | 5·8# ± 2·8 | 6·9 ± 3·2 |
| 360 | 37·7 ± 10·7 | 34·1# ± 17·9 | 35·6 ± 14·2 | 21·3 ± 2·4 | 12·8* ± 2·4 | 16·4 ± 5·1 |

# Arthritic = normal ($P > 0·1$). * Arthritic < normal ($P < 0·01$).

Table 3. Tissue uptake of i.v. [$^{125}$I]PVP (mean % accumulation of total dose ± s.d.).

| | Reticuloendothelial system | | Paws | | |
|---|---|---|---|---|---|
| PVP mol. wt (kDa) | Normal | Arthritic | Normal | Arthritic | Paw ratio Arthritic/normal |
| 10 | 0·28 ± 0·32 | 0·22# ± 0·29 | 0·13 ± 0·11 | 0·45* ± 0·31 | 3·5 |
| 40 | 0·00 | 0·00 | 0·07 ± 0·03 | 0·82* ± 0·22 | 11·7 |
| 360 | 5·99 ± 5·14 | 9·57# ± 6·82 | 0·23 ± 0·08 | 3·58* ± 1·43 | 15·6 |

# Arthritic = normal ($P > 0·1$). * Arthritic > normal ($P < 0·01$).

normal paw where uptake of 360 kDa PVP exceeded that of both 40 and 10 kDa PVP ($P < 0·01$) but no difference was seen between 40 and 10 kDa PVP. Uptake of PVP of mol. wt 360 kDa by arthritic paws was 10 times greater than that of 10 kDa, with only a 2-fold increase in uptake of the same preparation by normal tissues. In addition, uptake of 360 kDa PVP by arthritic paws exceeded that by normal paws by a factor of 7. When expressed as a percentage of the total dose administered (Table 3), 16 times as much 360 kDa PVP was captured by arthritic than by normal paws (3·6 and 0·2%, respectively), while uptake of 40 and 10 kDa PVP was negligible (< 1%) in all tissues.

Uptake of [$^{125}$I]PVP by the heart, thyroid, kidneys and stomach was also quantified with no significant differences found between normal and arthritic rats ($P > 0·1$). However, 360 kDa [$^{125}$I]PVP accumulated to a greater extent than the two lower mol. wt preparations in heart and thyroid ($P < 0·01$).

## Discussion

Before any studies of PVP distribution in rats could be undertaken, it was necessary to develop a satisfactory method of iodination and to assess stability of the radioisotope under experimental conditions. When the method used by Regoeczi (1976) was applied to iodinate PVP, the efficiency of labelling was found to be both inconsistent and markedly lower than the 20–25% efficiency previously reported. Therefore a modification of the radiolabelling technique was introduced whereby the labelled polymer was dialysed before adusting the pH. This minor alteration of the initial method was found to increase the labelling efficiency from a mean value of 6% to approximately 25% (unpublished data), the latter value being comparable with that obtained by Regoeczi (1976). Using this modification, the stability of the iodinated PVP preparations was also improved with only 3 and 4% free iodide released from 40 and 10 kDa PVP, respectively, after 3 h incubation in normal rat serum, and 14% iodide becoming disassociated from 360 kDa PVP after a 20 h incubation. The amount of activity lost by spontaneous deiodination following the use of the modified method of labelling was considered to be low enough to merit the use of $^{125}$I as in-vivo tracer of PVP.

Following confirmation of the stability of iodinated PVP, experiments were undertaken to investigate the in-vivo fate of the polymer. The more rapid clearance of high mol. wt PVP from the circulation of arthritic rats compared with normal animals ($P < 0·01$, terminal phase only) may possibly be attributed to the increased capture of the polymer by inflamed tissue and enlarged liver of the arthritic rats. The short initial half-lives of each PVP preparation was probably due to a relatively rapid penetration of the tissues and a relatively slow excretion of the polymer immediately after injection. The similar initial half-lives of 10 and 40 kDa PVP (6·1 and 6·9 min, respectively) were probably due to the similarity in size of these polymers enabling each to traverse the glomerular membrane for urinary excretion. The longer initial half-life of 360 kDa PVP (37·7 and 34·1 min for normal and arthritic rats, respectively) reflects its inability to cross the glomerular barrier. Once equilibrium between the tissues and blood had been reached then a slower terminal clearance phase was observed with a mol. wt-dependent decline of polymer concentration due to excretion. The half-life values are comparable with those in the literature; Ravin et al (1952) found that the half-lives of iodinated PVP in dog plasma were 1 h (28 kDa) and 3 h (35 kDa), while PVP of mol. wt 50 kDa had 75% remaining in the circulation after 12 h. In addition, Hulme et al (1968) have reported that [$^{125}$I]PVP with an average mol. wt of 100 kDa had a serum half-life of 12 h, and Ringsdorf (1975) has shown that a high percentage of 170 kDa PVP remained in the circulation 72 h after injection. Ringsdorf suggested that this may be due to high binding to transport proteins in the blood, but a more probable explanation is that PVP of mol. wt > 40 kDa cannot pass the glomerular membrane and therefore is not excreted in the urine (Ravin et al 1952). Munniksma et al (1980) have demonstrated the involvement of glomerular excretion of lower mol. wt polymers by comparing the clearance of a 33 kDa molecule by normal and nephrectomized rats. Clearance times were greatly impaired in the nephrectomized group while 55% of the injected dose was detected in the urine of normal rats within 24 h. On the basis of these results it is proposed that the different half-lives of [$^{125}$I]PVP preparations used was a direct consequence of the mol. wt-dependent glomerular excretion of this polymer. Owing to the trend for prolonged retention with increasing mol. wt of the polymer, corresponding enhanced tissue uptake was expected with higher molecular mass preparations.

Accumulation of [$^{125}$I]PVP by tissues of the reticuloendothelial system appeared to be proportional to the circulating terminal half-life, with little uptake of 10 and 40 kDa PVP (less than 0·1% (g tissue)$^{-1}$) reflecting the respective half-lives of 2·2 and 6·9 h. Conversely, 360 kDa PVP, which had a mean terminal half-life of 16·4 h, accumulated to a far greater extent (up to 1·7% (g tissue)$^{-1}$) in both normal and arthritic reticuloendothelial tissues ($P < 0·01$). A possible explanation is that the rapid elimination of 10 and 40 kDa PVP due to kidney filtration provides little opportunity for tissue accumulation, while the much larger 360 kDa PVP molecule, which is not filtered out in the urine and is thus retained in the circulation, may accumulate in tissues, particularly those of

the reticuloendothelial system. Our results are similar to those obtained by other workers who used large molecules of PVP (> 100 kDa) and showed accumulation in the reticuloendothelial system (Ravin et al 1952; Hulme et al 1968). Enhanced uptake of the polymer by the liver of arthritic rats over normal rats may be due to morphological changes in this tissue (granulomatous lesions and hepatomegaly) which are sometimes evident in adjuvant-induced arthritic rats (Pearson 1956).

Accumulation of PVP in both normal and arthritic paws was related to mol. wt. However, uptake by arthritic tissue greatly exceeded that by normal tissue for all mol. wts of PVP ($P < 0.01$). As data had already been corrected for any increased blood volume in the inflamed paws by using [$^{51}$Cr]RBC as a blood pool marker, the increased uptake was therefore due to the pathological condition of the tissue rather than to the effects of increased blood volume. Again, the 360 kDa PVP accumulated to a greater extent than the lower mol. wt preparations ($P < 0.01$). This effect was independent of the amount of circulating PVP (17% for 360 kDa) since a greater percentage of the 40 kDa polymer (34%) was present in the circulation at the time of death, in arthritic rats, but less tissue uptake of this preparation was observed. However, it is unclear if the increased paw uptake is due to endocytic capture of the polymer by infiltrating and resident phagocytes, or whether the increased escape of polymer at sites of inflammation due to increased capillary permeability is responsible for the enhanced accumulation.

When the data are expressed as percentage accumulation of the total dose administered, rather than (g tissue)$^{-1}$, less than 1% of 40 and 10 kDa PVP was captured by arthritic paws. This finding is similar to the accumulation of < 1% of i.v. injected microspheres within inflamed tissue reported by Illum et al (1989). However, almost 4% of injected 360 kDa PVP was associated with the inflamed tissues (compared with only 0·2% in normal tissues) which suggests an active accumulation of the polymer, possibly by macrophages and endothelial cells (Mizushima 1985).

The accumulation of PVP within rat inflamed paws reported here is similar to the findings of other workers. For example, De Schrijver et al (1987) found that i.v. injected nanocolloids were taken up at sites of inflammation but did not accumulate in normal tissues. The same distribution pattern has been reported with liposomes (Williams et al 1986; Love et al 1989), lipid microspheres (Mizushima 1985) and coated microspheres (Illum et al 1989).

Cellular uptake of polymers is probably executed by fluid phase endocytosis/pinocytosis which is initiated by adsorption to the cell membrane (Praaning-van Dalen et al 1981). High mol. wt polymers have been shown to have a higher rate of endocytosis than those of lower mol. wt (Ringsdorf 1975); this would also facilitate the increased tissue uptake of the 360 kDa PVP observed here. Therefore size of the polymer will not only control the plasma clearance kinetics but will also influence its tissue uptake profile. Hence despite there being similar levels of 40 and 360 kDa PVP in the circulation at the time of death, 35 and 25%, respectively (mean values), uptake of the latter greatly exceeds that of the former in all tissues of the reticuloendothelial system and inflamed paws ($P < 0.01$). However, the possibility that free iodide as well as PVP-associated iodine was taken up by inflamed paws cannot be ruled out, since 14% free iodide was shown to be released from the 360 kDa [$^{125}$I]PVP preparation in-vitro. Further studies on the in-vivo stability of [$^{125}$I]PVP are needed in order to determine if free iodide, which if released may be distributed to inflamed tissue, contributes to the amount of activity taken up by the paws.

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